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VOL. 4.

JULY-DECEMBER

**I**  
1923

# THE JOURNAL

OF

# METABOLIC RESEARCH

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Published by

THE PHYSIATRIC INSTITUTE  
Morristown, New Jersey, U. S. A.

PRICE: \$10.00 PER YEAR



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# AN APPARATUS FOR THE EXACT ANALYSIS OF AIR IN METABOLISM INVESTIGATIONS WITH RESPIRATORY EXCHANGE CHAMBERS\*

By THORNE M. CARPENTER

*(From the Nutrition Laboratory of the Carnegie Institution of  
Washington, Boston, Mass.)*

In one of the older methods of determining the respiratory exchange of men and animals, the subject was placed in a chamber into which room air or outdoor air was passed. Samples of outgoing air were then collected and analyses made on them. This method is illustrated by the Pettenkofer-Voit<sup>1</sup> apparatus into which air was introduced from out-of-doors, the carbon dioxide and water in samples of the air entering the chamber were determined by absorption, and then air was drawn out from the chamber by means of a meter, and an aliquot automatically drawn through absorbers for the collection of the water and carbon dioxide. In the Jaquet<sup>2</sup> method, the air is likewise drawn through the chamber, but the determinations of the carbon dioxide and oxygen are made by means of a gas-analysis apparatus. Thus, by using the measurements of the oxygen and carbon dioxide in the air entering and leaving the chamber, and the amount of air passing through the chamber, it is possible to find the change in composition and to calculate the carbon-dioxide elimination and oxygen absorption of the subject.

More recently a large chamber for group work has been designed by Professor Francis G. Benedict,<sup>3</sup> in which an aliquot of the outgoing air is conducted through absorbers which remove the carbon dioxide and water; the remainder of the air is discharged into the open room. The aliquoting is done by a special device in which the ratio between the amount rejected and the amount collected is determined experimentally. This, however, gives only a measure of the carbon dioxide in the air. If the air is analyzed at the same time, and the ratio between the carbon-dioxide increment and the oxygen deficit found, one can

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\* The apparatus was demonstrated and a preliminary abstract presented at the meeting of the Federation of American Societies for Experimental Biology at Toronto, Canada, December, 1922. (J. Biol. Chem., 55, 1923; Proc., p. xix.)



calculate from the total amount of carbon dioxide absorbed the amount of oxygen which has been used by the group in the chamber.

In all of these apparatus the ventilation must be at such a rate that the carbon dioxide in the outcoming air will not usually rise above 1 per cent. Accordingly, if the respiratory quotients vary between the normal limits, namely, 0.70 and 1.00, the oxygen deficit varies from 1.43 to 1.00 per cent.

At the present time the gas-analysis apparatus which are suitable for analyzing chamber air are the Pettersson apparatus used by Grafe<sup>4</sup> and by Gigon,<sup>5</sup> the Sondén<sup>6</sup> apparatus, and an apparatus devised by Krogh<sup>7</sup> for analyses to 0.001 per cent. For less exact work, the ordinary form of the Haldane<sup>8</sup> gas-analysis apparatus may be employed, but the limits of accuracy are not so great as with the more refined forms referred to above.

### *New Gas-analysis Apparatus*

The new gas-analysis apparatus here to be described was devised for use in an investigation of the metabolism of steers which is being carried out by Professor Francis G. Benedict, with the co-operation of Professor E. G. Ritzman, at the New Hampshire State Agricultural Experiment Station. In this investigation, a chamber<sup>9</sup> is employed which is constructed on the same principle as the group chamber previously referred to. The respiratory quotient is not determined by this method, but with a suitable gas-analysis apparatus, the ratio can be found by analyzing the air leaving the chamber. It seemed impracticable to attempt a duplication of the Krogh apparatus, and although the Nutrition Laboratory has a Sondén apparatus, it is too fragile to transport and very difficult of manipulation. Accordingly, at the suggestion of Professor Benedict, a new gas-analysis apparatus was designed.

The general principle of the new apparatus is that of the Haldane gas-analysis apparatus for expired air, modified to meet the necessary degree of refinement in reading and to make the apparatus more reliable and technically simpler to use. A diagrammatic sketch of the apparatus is given in figure 1.\* It consists of the following parts: The measuring burette, *A*; com-

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\* The glass parts were made by Emil Greiner Co., 55 Fulton St., New York City.

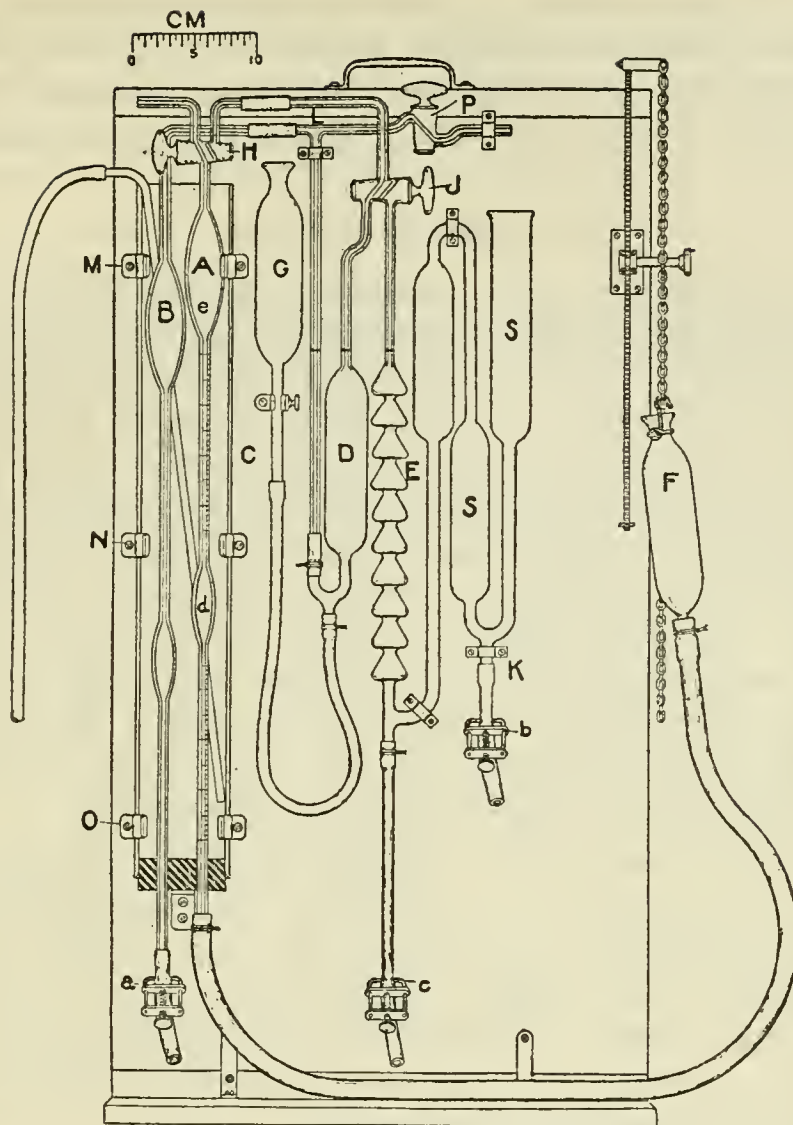


Figure 1

Fig. 1. *Diagram of Gas-analysis Apparatus*

The measuring burette, *A*, and the compensator, *B*, are immersed in water in the container, *C*. Communication between the burette, *A*, and the carbon-dioxide absorption pipette, *D*, and the oxygen absorption pipette, *E*, is secured by taps, *H*, and *J*, and between the compensator, *B*, and the pipette, *D*, by the capillary tee, *L*. The tap, *P*, provides communication with the open air in the preliminary adjustment of the apparatus. *F* is a mercury leveling bulb for the burette, *A*, and *G* is a leveling bulb for the pipette, *D*. *S, S* is a water reservoir with outlet *K*, and protects the reagent in *E* from the air, also serves as a pressure medium. Pinch-cocks *a*, *b*, and *c*, provide for the introduction or withdrawal of liquids. *M*, *N*, and *O* are brass spring clamps holding the water jacket and its contents in position.



pensator, *B*; water jacket, *C*; carbon-dioxide absorption pipette, *D*; oxygen absorption pipette, *E*; mercury leveling bulb, *F*; leveling bulb for the potassium hydroxide solution, *G*; water reservoirs, *S, S*; taps, *H, P*, and *J*; and capillary tee-tube, *L*.

### *Description of Parts*

*Measuring Burette.* The measuring burette, *A*, is a fundamental part of the apparatus and has been most modified to meet the requirements. It is constructed of heavy-walled glass. In the original construction a tap is fused on at the lower end for calibration purposes, but after calibration, this tap is removed. Like the burette in the Söndén apparatus, the measuring burette, *A*, is made up of two bulbs, *e* and *d*, and two graduated capillaries. Both bulbs taper at each end to provide smoothness and safety in operation. The upper bulb, *e*, retains the nitrogen remaining after the absorption of the carbon dioxide and oxygen, while the smaller bulb, *d*, provides for the major portion of the contraction due to the absorption of oxygen, and thus makes it possible to shorten the burette so that it will not be unwieldy.

The main qualification for high accuracy is the ability to determine exactly small divisions of volume. In this apparatus, as in the Haldane apparatus, the setting of the levels of the two absorbing solutions is very simple and errors of 0.1 or 0.2 millimeter are of no significance. Therefore, to increase the accuracy it is necessary to increase the degree of refinement with which the readings of the burette can be made.

The full capacity of the burette, i. e., from the tap *H* to the lowest graduation on the burette, is 40.04 cc. From the tap *H* to the first division below the upper bulb, *e*, the capacity is 31.36 cc. No portion of this tap is included in these capacity measurements, so corrections for this are not required in calibrations. From 31.36 cc. to 31.84 cc., the capillary is divided into 120 divisions, each corresponding to 0.004 cc. The second bulb, *d*, occupies the space between 31.84 and 39.36 cc. Between 39.36 and 40.04 cc., the capillary is divided into 170 divisions. Each division is about 1 mm. in length, so that estimations can be made to 0.1 mm., thus giving a measurement of 0.0004 cc. In other words, the readings are accurate to 0.001 per cent. The divisions of the burette are marked as percentages of the volume rather than for

actual capacity, which greatly simplifies the readings. Thus, the volumes from 31.36 to 31.84 cc. are marked on the capillaries as 78.4 to 79.6 per cent., and those from 39.36 to 40.04 cc. are marked from 98.4 to 100.1 per cent. Each 0.1 per cent. is marked with a long line and a numeral, the divisions for 0.05 per cent. have slightly shorter lines, and those for 0.01 per cent. are marked with still shorter lines.

The length between the tap, *H*, and the upper bulb, *e*, is 5 cm.; the length of the large bulb, *e*, is 9 cm.; the length of the small bulb, *d*, is 6 cm. The distance from 78.4 to 79.6 per cent. is 130 mm., and from 98.4 to 100.1 per cent is 181 mm., so that each 0.01 per cent. is slightly longer than 1 mm. The total length of the burette ready for use is 87 cm. The mercury in the burette is manipulated by the leveling bulb, *F*, which is attached to the bottom of the measuring burette by heavy-walled tubing.\*

*Compensator.* The compensator, *B*, duplicates the measuring burette, *A*, in construction and form. A short piece of rubber tubing connects it at the top with a capillary tee-tube, *L*, and a tap, *P*. At the bottom of the compensator is a piece of rubber tubing with a screw pinchcock (*a*) which can be opened when the compensator requires moistening.

*Water Jacket.* The water jacket, *C*, in which the measuring burette, *A*, and the compensator, *B*, are placed, is simply a cylindrical glass vessel which is held in place by means of brass spring clamps, *M*, *N*, and *O*.

*Absorption Pipettes.* The two absorption pipettes, *D*, for the absorption of carbon dioxide, and, *E*, for the absorption of oxygen, are attached directly to the same tap (*J*) without rubber connection. A Y-tube at the bottom of the absorption pipette, *D*, is connected by rubber tubing with the leveling bulb, *G*. The Y-tube is bent slightly backward so that the other arm can be connected with the tee-tube, *L*, of the compensator, *B*. The bulb of the absorption pipette, *D*, contains a 10 per cent. solution of potassium hydroxide for absorbing the carbon dioxide.

The absorption pipette, *E*, has a series of 10 bulbs which contain the potassium pyrogallate for the absorption of oxygen.

---

\* Pettersson (Zeitschr. f. analyt. Chem., 25, 1886, 467) used a glass stopcock to shut off the mercury from the leveling bulb after an approximate adjustment of the levels had been made, and carried out the final adjustment by means of a screw pinchcock around the rubber tube at the lower end of the burette. This method has been used in a number of apparatus and is now being tested with the apparatus here described.

Each bulb has a capacity of about 5 cc.; provision is thus made for all of the sample which can be drawn into the burette, *A*. This series of bulbs, which is adapted from Krogh's<sup>7</sup> gas-analysis apparatus, gives rapidity of oxygen absorption with no possibility of clogging, or trapping of gas bubbles, as sometimes occurs when glass tubes or rods are used.

The bulbs, *S*, *S*, when partially filled with water, provide a seal to protect the pyrogallate solution from the air. An outlet, *K*, at the bottom, gives opportunity for withdrawal of the water as desired.

*Capillary Tee-tube.* The capillary tee-tube, *L*, which connects the compensator, *B*, with the potassium hydroxide container, has been slightly altered from the form used in the ordinary Haldane apparatus, for at times the tap which is commonly employed has caused trouble due to the clogging of the ports by a slight film of grease. This interfered with the delicacy of adjustment of the final level. The tap, *P*, was therefore placed at the end of the arm opening to the outside air instead of at the central point of the tee. This permits the opening and closing of the tap to the outside air without danger of clogging, thus leaving the compensating bulb always open to the potassium hydroxide. The modification has proved very satisfactory.

*Taps.* The taps, *H*, *J*, and *P*, are all of the Greiner-Friedrich type. As the openings are set at an angle to one another, there is no danger of grooving from one port to the other, and the passages are always plainly visible so that it is possible to see which way the tap is turned.

*Rubber Tubing.* The connections between the glass parts are made by means of pure gum rubber tubing.\* The only exception is the connection between the mercury leveling bulb, *F*, and the measuring burette, *A*, where heavy-walled tubing is used of such a character that the mercury does not collect dirt.\*\*

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\* No. 4836, Scimatco rubber tubing, Scientific Materials Company, Pittsburgh, Pa.

\*\* No. 8842, nitrometer tubing, Arthur H. Thomas Company, Philadelphia, Pa. In order to prevent the accumulation of dirt from the rubber tubing by the mercury, Asher (Amstad, Biochem. Zeitschr., 145, 1924, 170) has installed in the Haldane apparatus an inverted Orsat pipette between the rubber tubing of the leveling bulb and the lower end of the burette. It is of sufficient size so that the mercury in the burette cannot pass into the rubber tubing of the leveling bulb, but enters the Orsat pipette. Liljestrand (private communication) has connected a spherical bulb below the burette for the same purpose.



### *Directions for Use*

Directions for the set-up and use of the apparatus are given in the following pages. Many of these details have been mentioned by various writers<sup>10</sup> in connection with the manipulation of the Haldane gas-analysis apparatus and the modifications by Henderson and Bailey,<sup>11</sup> but they will bear repetition here.

### *Calibration of the Measuring Burette*

As previously stated, the burette, *A*, in its original construction, has a one-way tap fused on at the bottom, which forms a part of the burette. This tap is for use in calibration and has no permanent place in the apparatus.

For calibration, it has not been found necessary to enclose the burette in a water jacket, but it is simply set up on a retort stand and firmly fixed in position by means of burette clamps attached at two points. An evaporating dish, partly full of clean mercury, is held under the point of the calibration tap, and suction is applied at the end of a rubber tube attached to the arm above the upper tap, *H*. The mercury is thus drawn up through the calibration tap into the burette, through the tap, *H*, and into the arm to which the rubber tube is attached. As the burette is long, the mercury may be more easily drawn up by inclining the burette towards the suction attachment. Care must be taken, however, to exclude completely all bubbles of air as the mercury is drawn up through the burette. When the burette is full, the calibration tap is closed and a few minutes are allowed to elapse until temperature equilibrium has been obtained throughout the mass of mercury and the glass walls of the burette. A series of weighed bottles should be ready at hand, and sufficient in number to provide for the entire calibration, so that there will be no interruption in the process of drawing off the mercury.

When all is in readiness for drawing the mercury, the tap, *H*, is reversed; the volume of mercury then represents the volume of the burette, since no part of the stop-cock enters into the capacity of the burette. The mercury is drawn down into a weighing bottle until the level is at approximately the first mark on the upper capillary. An exact reading is now made to 0.001 per cent., and recorded. As soon as possible, another section of mercury is drawn and the reading recorded. These readings are con-

tinued down to the last graduation on the lower capillary. It has been found advisable for each drawing of mercury to approximate 0.1 per cent., except when the lower bulb, *d*, is reached, where the drawing is from the last graduation above the bulb to the first graduation below it. To promote rapid drawing, it is desirable that one person should pass the weighing bottles to the observer, who draws the mercury and makes the reading, which is recorded by the assistant. After the whole series of drawings has been completed, the bottles are again weighed, the differences between the weights of the empty and filled bottles being, of course, the weights of the mercury which have been drawn from the individual sections of the burette. The calculation of the corrections to be made is carried out as follows:

The weight of the mercury first drawn, i. e., that from the section between the tap, *H*, and the first graduation, or from 0 to 78.4 per cent., is taken as the standard for the whole burette and the weight of the mercury per unit of volume is calculated from this. The difference between the second and third readings gives the apparent volume of the second section. The true volume is calculated by dividing the weight of mercury drawn from this section by the weight per unit of volume previously determined from the weight of mercury in the first section. The volumes for the individual sections are calculated separately, but the correction table is made up by taking the first volume as the basal unit, adding to that the actual volume calculated for the second section, and comparing it with the volume read, the difference giving the correction to be applied to the reading for the second section. The corrections for the subsequent sections are calculated in the same way, the calculated volume for each interval being added to the calculated total volume for the previous sections.\* The percentage volumes are totalled rather than the weights of mercury, as errors in individual calculations are thus corrected without the necessity for the entire recalculation of each volume following the error.

A sample set of weighings, calculations, and calibration corrections is given in Table I. In general, it has been found that the corrections for the burettes have been in the nature of a straight

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\* The apparent slight discrepancies in the column headed, "Calculated total volume," in Table I, are due to the fact that the fourth decimal figure is not recorded in this and the preceding columns.



line, but in one case a very distinct curve was found and subsequent analyses of outdoor air and of air leaving an alcohol flame showed these corrections to be accurate. After several well-agreeing calibrations have been obtained, they are charted and a table is drawn off from the chart. The lower tap is then severed from the burette.

TABLE I

Example of Calibration of the Measuring Burette of the Gas-analysis Apparatus

Burette Readings	Weight of Mercury	Calculated Volume of Section	Calculated Total Volume	Correction
	gm.	cc.	cc.	cc.
0 — 78.400	423.6934 <sup>1</sup>	-----	-----	0.000
78.400 — 78.506	0.5533	0.102	78.502	— .004
78.506 — 78.610	.5572	.103	78.606	— .004
78.610 — 78.712	.5482	.101	78.707	— .005
78.712 — 78.814	.5397	.100	78.807	— .007
78.814 — 78.916	.5438	.101	78.907	— .009
78.916 — 79.019	.5555	.103	79.010	— .009
79.019 — 79.129	.5967	.110	79.121	— .008
79.129 — 79.234	.5658	.105	79.225	— .009
79.234 — 79.337	.5548	.103	79.328	— .009
79.337 — 79.468	.7130	.132	79.460	— .008
79.468 — 98.417	102.2774	18.925	98.385	— .032
98.417 — 98.518	.5310	.098	98.483	— .035
98.518 — 98.619	.5281	.098	98.581	— .038
98.619 — 98.736	.6085	.113	98.694	— .042
98.736 — 98.842	.5773	.107	98.801	— .041
98.842 — 98.955	.5950	.110	98.911	— .044
98.955 — 99.056	.5422	.100	99.011	— .045
99.056 — 99.161	.5577	.103	99.114	— .047
99.161 — 99.266	.5540	.103	99.217	— .049
99.266 — 99.359	.4988	.092	99.309	— .050
99.359 — 99.469	.5862	.109	99.417	— .052
99.469 — 99.564	.5153	.095	99.513	— .051
99.564 — 99.676	.5953	.110	99.623	— .053
99.676 — 99.780	.5694	.105	99.728	— .052
99.780 — 99.888	.5756	.107	99.835	— .053
99.888 — 100.031	.7715	.143	99.978	— .053
100.031 — 100.100	.3845	.071	100.049	— .051

<sup>1</sup>The weight of mercury between 0 and 78.400 is taken as the standard for the remainder of the burette. Dividing this weight by 78.400 gives 5.4043 grams, which is taken as the weight per unit of volume.

#### *Preparation of Apparatus for Use*

After a satisfactory calibration of the burette, the apparatus is put in order for use. The tubing connecting the mercury reser-

voir, *F*, with the bottom of the burette, *A*, must be wired, also all tubing attached to the absorption pipettes which may come in contact with the liquids, as the alkaline solutions are apt to make the pure gum tubing slippery. It does not appear to be necessary to wire the tubing at the points where the burette and the compensator, *B*, are attached.

The compensator, *B*, must be moistened. This is readily done by opening the screw pinchcock, *a*, and drawing water through the tubing and bulbs up to the elbow at the top above the water jacket. In doing this, however, it is necessary to close the rubber tubing connecting the leveling bulb, *G*, with the absorption pipette, *D*, as otherwise the liquid or air in this container will be drawn up through this opening, and water will not be drawn into the compensator, *B*. When the upper bulb of the compensator, *B*, is full, the water should be allowed to drain gradually until it is near the bottom, though still visible. It should then be stopped by closing the pinchcock, *a*. It is absolutely necessary, however, that all bubbles of air are removed from the water. In earlier testing of the apparatus, considerable unnecessary trouble was experienced on account of the inclusion of a tiny bubble of air which, acting as a second manometer, caused inaccurate readings, although duplicates were obtained.

The carbon-dioxide absorption pipette, *D*, is next filled with a 10 per cent. solution of potassium hydroxide, taps *P* and *J* having previously been removed. Here again, it is absolutely necessary that bubbles of air be excluded. The simplest way to accomplish this is to lower the leveling bulb, *G*, somewhat below the Y-tube and pour the solution into the bulb gradually, raising and lowering *G* until the liquid is certainly free from all air bubbles. The raising and lowering should be done slowly, as otherwise the potassium hydroxide will be forced rapidly up into the capillary tee-tube, *L*, connecting with the compensator, *B*. If this happens, it is very difficult to remove it completely. A safe rule to follow is never to allow the solution to rise more than half way up the tube to the tee at *L*. The solution of potassium hydroxide used should be perfectly clear, which may be secured by filtering the liquid through glass wool.

The oxygen absorption pipette should then be filled with potassium pyrogallate made according to the formula of Haldane, i. e.,

10 grams of pyrogalllic acid dissolved in 100 cc. of a nearly saturated solution of potassium hydroxide having a specific gravity of 1.55.\* The filling of the pipette is most conveniently done if the reservoirs, *S, S*, are first partially filled with water. This supplies a pressure which assists in the proper adjustment of the level of the pyrogallate in the pipette. With the water present in the reservoir, *S, S*, and the tap, *J*, at the top of the pipette closed, the pyrogallate is introduced through a long stem glass funnel attached to the rubber tubing at the bottom of the pipette. This forces up the level of the water in the reservoirs. The tap, *J*, may next be gradually opened and the reagent allowed to come to its own level. It is then closed off, more pyrogallate added, and the process repeated until the solution is at the level desired. There is at first some difference in level between the pyrogallate above the bulbs and that in the right hand limb of the pipette, but after a time the oxygen from the air, which is enclosed between the solution and the water seal in the reservoirs, *S, S*, will be absorbed and the levels may be adjusted by the addition of more water. If the pressure is too great, water may be withdrawn at *K*, through the pinchcock, *b*, below the reservoirs.

The taps, *H, J*, and *P*, should be greased. If the filling of the potassium hydroxide pipette and the potassium pyrogallate pipette has been properly done, the rise of the reagents in the two pipettes will not be sufficient to cause trouble if the tap, *J*, between the two is removed. The lubricating grease used should be very smooth, and not stringy, or otherwise it will drag across the openings during use and possibly form a film, which will partly or completely occlude the ports. The tap is first lightly filmed over with the grease, then replaced in the barrel and turned around until it is seen that the surface of the tap is completely and evenly covered. The tap is then again removed and the ports, both in the tap and in the barrel, are cleaned, as they may have become partially filled up in this process.

In filling the leveling bulb, *F*, and the measuring burette, *A*, with mercury, care should be taken that both are dry, as well as the tubing connecting the two, otherwise the mercury is likely

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\* A series of analyses of outdoor air with this apparatus has shown that the potassium pyrogallate can be made up from potassium hydroxide purified with alcohol and that it can be used at once. Example: January 4, 1923. Solution of potassium pyrogallate (Kahlbaum's pyrogalllic acid; Kahlbaum's potassium hydroxide purified with alcohol), freshly made in the morning. Oxygen percentage: 11:15 a. m., 20.939 per cent.; 1:50 p. m., 20.943 per cent.; 2:30 p. m., 20.942 per cent.



to accumulate dirt much more quickly. By means of a medicine dropper or a fine pipette, water is then introduced at the opening of the left-hand bend above the burette, *A*, and the mercury raised and lowered several times to make sure that the inner surface of the burette is thoroughly wet. The excess water is rejected by removing the tap, *H*, and raising the mercury level until the excess water can be absorbed with a small piece of filter paper introduced into the opening. This water should always be removed, as it is liable to give trouble in analyses by clinging to the walls of the burette as the mercury passes by it, thus forcing up the level of the mercury and causing too low readings. If an excess amount of water is on top of the mercury, a smaller amount of gas is analyzed than is actually read, and consequently the results obtained are too low.

### *Preliminary Analysis*

To get the apparatus into working condition, a preliminary analysis of an air sample should be made before the regular air analysis is begun. This, while not giving quantitative results, serves to test the apparatus for leaks and demonstrates whether it is possible to obtain duplicate readings and uniformity in setting. For such an analysis, the burette, *A*, must be nearly full of air. To fill *A*, open tap, *H*, to the outside air and lower the mercury leveling bulb, *F*, until the burette is practically full, then reverse the tap, *H*, so that it will be in connection with the pipettes. The level over the potassium pyrogallate in the pipette, *E*, is then set by means of the leveling bulb, *F*. The tap, *J*, is next reversed so that it is open to the carbon-dioxide pipette, *D*, instead of to the pipette, *E*. The levels of the potassium hydroxide in *D* and *L* are now set by using both of the leveling bulbs, *F*, and *G*, with the tap, *P*, open. After these levels have been set, the tap, *P*, is closed and need not be opened again until the completion of a series of analyses. It should never be opened when the tap, *H*, on the burette, *A*, is turned to the outside air.

In the initial use of an apparatus, it is desirable to follow out the whole routine, first removing the carbon dioxide from the air and making a reading, then repeating the reading to determine

whether this part of the apparatus is air-tight and it is possible to duplicate readings. To remove the carbon dioxide completely, the mercury in the burette, *A*, is raised nearly to the tap, *H*, then lowered, this routine being carried out six times. After the levels in the pipette, *D*, and the tee-tube, *L*, have been again set as previously described, a reading is made. The procedure is then repeated. Usually complete absorption is obtained in the first routine of six movements, but the repetition supplies duplicate readings under like conditions. If the apparatus is functioning perfectly and the potassium hydroxide solution is new, there will be no difficulty in duplicating the readings exactly to within 0.001 per cent. If this accuracy is not obtained, the cause should be searched for.

The next process is to remove the oxygen from the air sample. The passage to the pyrogallate solution is opened by turning the tap, *J*, and the mercury bulb, *F*, is raised and lowered 10 times. The tap, *J*, is then reversed and the gas is driven twice into the potassium hydroxide pipette, *D*, after which the level of the reagent is again brought approximately to the mark in *D*. The tap, *J*, is then turned to connect with the series of bulbs in the pipette, *E*, and the gas is driven into the potassium pyrogallate solution five times, once more into the potassium hydroxide solution, and finally five times into the pyrogallate solution, tap *J* being reversed between each series of movements. Repeated attempts to shorten this procedure have been without avail, and if this routine is faithfully carried out and the potassium pyrogallate is efficient, the results obtained will be uniform.\* The level of the pyrogallate solution is now carefully adjusted to the mark, the tap, *J*, reversed, the gas driven twice into the potassium hydroxide, the level of the potassium hydroxide adjusted, and a reading made on the burette, *A*. The gas is then driven once into the potassium hydroxide solution, at least five times more into the pyrogallate solution, and twice into the potassium hydroxide solution, for the gas remaining may be so small in amount that a less number of times may not insure complete absorption. Another reading is now made which should agree within 0.002 of the previous reading.

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\* Siven<sup>12</sup> has reported a special absorption pipette in which he claims that the oxygen is completely absorbed out of 100 cc. of air by driving the air through the solution three times, but the pipette has not been tried with this apparatus.

The movements necessary for this preliminary analysis of an air sample may be summarized as follows:

Turn tap, *H*, to outside air and fill burette, *A*, with air by lowering mercury leveling bulb, *F*.

Reverse tap, *H*, and turn tap, *J*, to connect *A* with *E*.

Set the level in the potassium pyrogallate pipette, *E*, using leveling bulb, *F*.

Reverse tap, *J*.

Set levels in potassium hydroxide pipette, *D*, and tee-tube, *L*, using both leveling bulbs, *F*, and *G*, with tap, *P*, open.

Close tap, *P*.

The carbon dioxide in the air sample is first removed.

Raise mercury in burette, *A*, nearly to tap, *H*, then lower, 6 times in all.

Set levels in potassium hydroxide pipette, *D*, and tee-tube, *L*.

Make reading on burette, *A*.

Repeat routine of raising and lowering mercury 6 times, setting levels, and making reading. The carbon dioxide should now be completely absorbed as shown by the fact that the second reading duplicates the first record.

To remove oxygen, turn tap, *J*, to make connection with potassium pyrogallate pipette, *E*.

Raise and lower mercury bulb, *F*, 10 times.

Reverse tap, *J*.

Drive gas 2 times into the potassium hydroxide pipette, *D*.

Turn tap, *J*, to connect with potassium pyrogallate pipette, *E*.

Drive gas into potassium pyrogallate solution 5 times.

Reverse tap, *J*.

Drive gas into potassium hydroxide 1 time.

Reverse tap, *J*.

Drive gas into potassium pyrogallate solution 5 times.

Set level in potassium pyrogallate pipette.

Reverse tap, *J*.

Drive gas into potassium hydroxide 2 times.

Set level in potassium hydroxide pipette.

Make reading on burette, *A*.

Drive gas into potassium hydroxide solution 1 time.

Reverse tap, *J*.

Drive gas into potassium pyrogallate solution 5 times.

Set level in potassium pyrogallate pipette.



Reverse tap, *J*.

Drive gas into potassium hydroxide 2 times.

Make reading on burette, *A*.

The oxygen should now be completely absorbed, as indicated by satisfactory agreement of second reading with first reading.

If one begins the analysis with all parts of the apparatus full of ordinary air, and the volume in the measuring burette at about 100 per cent., the contraction due to the absorption of oxygen will be greater than if one analyzes only the volume of air in the burette itself, because the capillaries also contain oxygen. The contraction may then be so large that when the attempt is made to bring the pyrogallate solution to the line in the pipette, *E*, the amount of gas remaining may not be sufficiently large to read or even to fill the large bulb, *e*. This is a dangerous condition, for the downward movement of 1 mm. of mercury in the bulb, *e*, of the measuring burette, *A*, may lead to the drawing up of the pyrogallate solution into the capillaries above the tap, *J*, as the cross section of the bulb is very large in comparison with that of the capillary tubing. To avoid such a contingency, the leveling bulb, *F*, should be hung upon the chain connected with the rack and pinion, then gradually lowered until the pyrogallate solution nearly fills the uppermost bulb in the pipette, *E*. The tap, *H*, is next reversed so that connection is made with the outside air, and air is drawn in by lowering the mercury leveling bulb, *F*, until the volume of gas in *A* reaches the lowest graduation between bulb, *e*, and bulb, *d*. The tap, *H*, is again reversed so that connection is made between the burette, *A*, and the pyrogallate pipette, *E*, and the absorption of oxygen may then continue as usual. The nitrogen in the portion of air added will nearly always suffice to give enough residual gas so that readings can be made after the oxygen has been completely absorbed. In making a preliminary analysis, it should never be assumed that all the oxygen is absorbed unless two successive readings can be obtained which demonstrate this.

In case the residual volume of gas is so large that it enters the lower bulb, *d*, a similar procedure can be used, although in this case it is better to discharge gas through the tap, *H*, into the outside air until the level of the mercury rises approximately to the lower part of the graduated stem between bulbs, *d*, and *e*, in the measuring burette.

*Regular Analysis of Air Sample*

The apparatus is now ready for the regular analysis of air samples. Without altering the levels in any way or opening the tap, *P*, to the air, the tap, *H*, on the burette is turned so that connection is made with a sampling device or with the outside air. Either of two methods may be used for drawing the sample: (1) The displacement method used by Haldane, in which all of the capillaries and connecting tubes are filled with mercury; or (2) the washing method, in which portions of the air samples are repeatedly drawn and discharged until all the connections are thoroughly washed out with the gas to be analyzed. The washing method appears to be preferable for this apparatus. According to the custom used in this Laboratory, a sufficient portion of the air sample is drawn into the burette, *A*, to fill the bulb, *e*, about one-third full; it is then rejected into the open air. This process is twice repeated, making three washings in all.

When the washing method is used, care must be taken to prevent "back lash," that is, a vibration of the mercury when it is forced up into the capillary above the upper bulb, *e*. If this occurs at the moment when the tap on a sampler is reversed, some of the air which has been rejected in the washing method may be drawn back into the capillary. A method which has been found to give good results is the use of a Greiner-Friedrich stopcock or tee-tap, the air being rejected through one free end of the tap. If a capillary tube of about 12 cm. in length is attached to this, air from the room cannot then get into the tap, as the attached capillary tube will be filled with the air just rejected. The last washing of air should have the same composition as that of the sample, consequently any backward movement of air in the capillary tube will not affect the composition of the sample drawn through the tap on the sampler.

After the washing out has been finished, the sample is drawn down to about 100 per cent., and adjustment is made with the outside air so that the gas is nearly at atmospheric pressure. The tap, *H*, is then reversed, thus connecting the burette, *A*, and the potassium hydroxide pipette, *D*. By raising and lowering the mercury reservoir, *F*, and the leveling bulb, *G*, the levels in the potassium hydroxide pipette, *D*, and the capillary tee-tube, *L*, are adjusted accurately in their original positions. After the gas



has been introduced into the burette, sufficient time must be allowed for a complete saturation of the air sample with moisture and for temperature equilibrium between the gas in the burette and the water bath. A good practice to follow is to set the levels, make the reading, and record it, then return to the apparatus and note if the levels have remained constant. If not, another adjustment should be made, and this routine repeated until two successive readings have been obtained which are identical within 0.001 per cent. The removal of the carbon dioxide and the oxygen from the sample may then be made in the manner described for the preliminary analysis. A complete analysis of an air sample requires about 30 minutes. The pyrogallate pipette holds about 75 cc. of solution, which is sufficient for 10 analyses.

#### *Precautions Regarding Use of Apparatus.*

In the use of this gas-analysis apparatus, it has been found that erroneous results, when obtained, were frequently due to the lack of observance of one or more of the following rules which apply to all forms of gas-analysis apparatus constructed on the Haldane principle. These are as follows:

1. The water jacket should contain sufficient water to more than cover the upper bulb of both the compensator, *B*, and the measuring burette, *A*. Slight changes in temperature of the water and outside air will then act uniformly upon the gases in both vessels.

2. The water in the water jacket should be vigorously stirred when the levels are set and read. This may preferably be accomplished by running a current of compressed air through the water in the vessel during an analysis. No attention need then be paid to this factor in the manipulation of the apparatus.

3. There should be enough water in the measuring burette, *A*, to insure a definite water meniscus when the mercury is raised to the upper part of the graduated capillary between bulbs, *d* and *e*.

The levels in either the potassium hydroxide or the potassium pyrogallate solutions should respond to the slightest change in level of the mercury bulb. Even though the gases may be driven back and forth by large changes in the position of the mercury leveling bulb, *F*, accurate results cannot be obtained unless the openings of the taps are free enough so that the liquids will

respond to the slightest change in the level of the mercury container. If this is not possible, either the grease has clogged the openings of taps, *H* or *J*, or a globule of mercury is caught in tap, *H*, of the burette. In either case the condition should be remedied immediately.

Successive readings after the presumably complete absorption of carbon dioxide or oxygen should never show an increase in volume of more than 0.002 per cent. If a larger increase is obtained in the removal of carbon dioxide from outdoor air (when the contraction would be 0.030 per cent.), it indicates that the saturation of the air in the original volume was not complete. This may be due to too brief a period between the introduction of the gas into the burette and the reading of the initial volume, or by insufficient water in the burette. In the latter case, additional moisture is absorbed by the gas when it is driven into the potassium hydroxide. If the increase in volume occurs during the analysis of a regular air sample, it is due to a bubble of liquid in some connection between the burette, *A*, and the pipettes, or to partly occluded ports of the taps. Successive readings should show either a decrease in volume or a constant volume within 0.002 per cent. It has been our experience that final readings can be duplicated to 0.001 per cent.

Theoretically an increase in readings may occur as the result of a leak, but in practice this is rarely found to be the case. There should be no changes in level of the pyrogallate while the gas is being passed into the potassium hydroxide and *vice versa*. If changes occur, it is due to leaks around the tap, *J*. Such a leak does not necessarily mean loss of air but usually change from one capillary to another, and accurate analyses under these conditions are difficult to obtain.

When a series of analyses is finished, the tap, *P*, on the compensating bulb should be left open to the air, and the other two taps (*H* and *J*) should be closed. Then no changes in levels can take place, and if by accident there is a break in the tubing connecting the mercury reservoir, *F*, with the burette, *A*, no harm can come to the apparatus.

Whenever a change is made in the apparatus, such as the putting on of new tubing, taking out and greasing the taps, or the introduction of new solutions, its efficiency should be tested by control analyses of outdoor air. Even though the apparatus has

functioned perfectly before such changes were made, no regular analyses should be conducted without these tests.

It is very difficult to obtain analyses on a day with variable winds or during a thunderstorm because of the extremely sudden and marked changes in atmospheric pressure.

Samples of gases can be analyzed several days after collection provided they are dried by passing them through sulphuric acid and collected over mercury.

*Example.* Sample collected February 14, 1924. Analyses: February 15, CO<sub>2</sub>, 0.572; O<sub>2</sub>, 20.237. February 16, CO<sub>2</sub>, 0.574; O<sub>2</sub>, 20.226. February 18, CO<sub>2</sub>, 0.575; O<sub>2</sub>, 20.230. February 21, CO<sub>2</sub>, 0.570; O<sub>2</sub>, 20.237.

### *Results Obtained With the Apparatus*

Analyses of outdoor air made at various times by three analysts are given in table II and indicate the general character of the results which one may expect to obtain. In general it is required that each individual analysis shall give  $0.030 \pm 0.003$  per cent. for carbon dioxide, and  $20.940 \pm 0.005$  per cent. for oxygen with samples of outdoor air. With few exceptions the results obtained by the three analysts come within these ranges.

In a previous publication,<sup>13</sup> it was pointed out that while an analysis of outdoor air served as a test of the whole apparatus, we had no method of controlling the apparatus with analyses of air similar in composition to that obtained under experimental conditions. For a complete test it would be desirable to have a change in composition comparable to that which would occur in actual metabolism studies. Such conditions can be obtained by use of an ethyl alcohol flame enclosed in a chamber (either of metal or glass), through which pure outdoor air can be drawn at such a rate that the desired percentage change can be secured. A number of such trials were made with an alcohol flame enclosed in a special lamp.<sup>14</sup> The results are given in table III. In such tests it is desirable to have increments in carbon dioxide of not less than 0.5 per cent. and deficits in oxygen of not less than 1.0 per cent.



TABLE II  
Results of Analysis of Outdoor Air

Date	CO <sub>2</sub>	O <sub>2</sub>	Analyst	Date	CO <sub>2</sub>	O <sub>2</sub>	Analyst
1922	%	%		1922	%	%	
June 28	0.031	20.941	E. L. F.	Nov. 11	0.029	20.942	L. A. R.
" 28	.032	20.945	"	" 12	.030	20.940	"
" 28	.030	20.938	"	" 13	.029	20.948	"
" 28	.032	20.935	"	" 14	.031	20.937	"
" 28	.032	20.944	"	" 15	.030	20.938	"
" 28	.031	20.937	"	" 16	.030	20.945	"
" 28	.030	20.940	"	Dec. 12	.029	20.942	"
July 19	.032	20.946	M. L. B.	" 13	.029	20.934	"
" 19	.031	20.940	"	" 18	.031	20.940	"
" 19	.032	20.944	"	" 19	.030	20.942	"
" 21	.032	20.941	"	" 21	.032	20.942	"
Sept. 26	.027	20.939	E. L. F.	" 27	.029	20.943	"
" 26	.031	20.941	"	" 30	.031	20.937	"
" 26	.029	20.944	"	1923			
" 26	.034	20.940	"	Jan. 4	.039	20.939	E. L. F.
" 26	.030	20.939	"	" 4	.030	20.943	"
" 26	.031	20.935	"	" 4	.029	20.942	"
" 26	.029	20.942	"	" 5	.029	20.945	L. A. R.
" 26	.031	20.943	"	" 6	.030	20.946	"
" 28	.029	20.941	"	" 6	.031	20.936	E. L. F.
" 29	.035	20.938	"	" 6	.029	20.943	"
Oct. 7	.030	20.943	L. A. R.	" 6	.032	20.940	"
" 7	.030	20.944	"	" 6	.030	20.949	"
" 9	.031	20.933	"	" 6	.033	20.937	"
" 9	.030	20.944	"	" 6	.031	20.935	"
" 10	.031	20.938	"	" 6	.031	20.944	"
" 10	.032	20.947	"	" 8	.029	20.940	"
" 10	.031	20.943	"	" 8	.030	20.944	"
" 12	.029	20.938	"	" 8	.030	20.936	"
" 17	.029	20.943	"	" 8	.032	20.941	"
" 18	.030	20.940	"	" 10	.031	20.937	L. A. R.
" 20	.028	20.943	"	" 11	.031	20.938	"
" 28	.029	20.941	"	" 12	.030	20.938	"
Nov. 1	.030	20.946	"	" 12	.029	20.939	"
" 2	.029	20.944	"	" 19	.031	20.941	"
" 7	.031	20.950	"				
" 8	.029	20.942	"				
" 9	.030	20.939	"				
" 10	.030	20.944	"				

TABLE III

Change in Composition of Outdoor Air Passing Over an Ethyl Alcohol Flame

[Theoretical R.Q. = 0.667]

Date	CO <sub>2</sub> Increase	O <sub>2</sub> Deficit	R. Q.	Analyst.
1922	%	%		
July 22	0.638	0.970	0.658	M. L. B.
" 22	.665	1.002	.664	"
" 22	.664	1.012	.656	"
Sept. 27	.792	1.193	.664	E. L. F.
" 27	.780	1.180	.661	"
" 28	.728	1.121	.649	"
" 28	.731	1.102	.663	"
Oct. 11	.537	.825	.651	L. A. R.
" 11	.451	.697	.647	"
" 11	.489	.753	.649	"
" 12	.551	.845	.652	"
" 12	.512	.773	.662	"
" 12	.358	.532	.673	"
" 16	.490	.729	.672	"
" 16	.639	.944	.677	"
" 16	.531	.782	.679	"

*Analyses Theoretically Possible With the New Gas-Analysis Apparatus.*

When the dimensions of the burette were being calculated, particularly those of the capillary sections, it was recognized that the range in the readings of the nitrogen residual should be wide enough to include all the variations which may be obtained in the changes of air occurring in ordinary respiration experiments with a chamber apparatus. The nitrogen residual of outdoor air is 79.03 per cent. If the respiratory quotient is below 1.00, the percentage of nitrogen rises above 79.03 per cent. When the respiratory quotient is over 1.00, as when carbohydrate is transformed into fat, the nitrogen residual is below 79.03 per cent. It seemed desirable to have possible a deficit in oxygen of 2.00 per cent., with a percentage of carbon dioxide of 1.50 per cent. or more. To give ample provision for these limits, the burette has been designed to analyze air containing 1.70 per cent. of carbon dioxide, with a residual nitrogen varying from 78.4 to



79.6 per cent. That analyses of air samples with such widely varying composition can be made is shown by the computations given in table IV.

TABLE IV  
Theoretical Analyses Which Can be Made with the New Gas-Analysis Apparatus

Theoretical Analysis			Change From Outdoor Air <sup>1</sup>		R. Q.
CO <sub>2</sub>	O <sub>2</sub>	N <sub>2</sub>	CO <sub>2</sub> Increase	O <sub>2</sub> Deficit	
%	%	%	%	%	
1.034	19.594	79.372	1.004	1.437	0.699
1.035	19.538	79.427	1.005	1.507	0.667
1.522	19.841	78.637	1.492	0.995	1.499
1.539	18.954	79.507	1.509	2.112	0.714

<sup>1</sup>Composition of outdoor air is taken as:  
CO<sub>2</sub>, 0.030; O<sub>2</sub>, 20.940; N<sub>2</sub>, 79.030

*Method of Calculating Gaseous Exchange from an Analysis of Chamber Air*

For purposes of discussion, it may be assumed that 100 volumes of air entering the chamber contain 0.030 volume of carbon dioxide, 20.940 volumes of oxygen, and 79.030 volumes of nitrogen, and that in its passage through the chamber, the animal has added 1.000 volume of carbon dioxide and taken from it 1.429 volumes of oxygen. This would give a respiratory quotient of 0.700. The volumetric composition of the outgoing air under these conditions would be as follows: Carbon dioxide, 1.030 volumes; oxygen, 19.511 volumes; and nitrogen, 79.030 volumes; making a total of 99.571 volumes of outgoing air. An analysis of the ingoing air would give, in percentage, the same proportions as those cited above, viz., carbon dioxide, 0.030 per cent.; oxygen, 20.940 per cent., and nitrogen, 79.030 per cent. The percentage composition of the outgoing air, however, is not represented by the figures given above for the volume of the air after it has passed through the chamber, as the total outgoing volume of air is less than that which entered the chamber, while the volume of nitrogen has remained unchanged. The percentage composition of this air would thus be: Carbon dioxide, 1.034 per cent.; oxygen, 19.595 per cent., and nitrogen, 79.371 per cent.

When the volumes of the ingoing and outgoing air and the percentage composition are obtained, the carbon-dioxide elimination and oxygen absorption of the animal can readily be calculated. Rarely, however, are all these data available as usually only the volume of the outgoing air and the percentage composition as determined by analysis are known. The oxygen absorption cannot therefore be calculated by difference. Under these circumstances, the method of calculation is as follows:

While the *volume* of the nitrogen has remained constant in the air in its passage through the chamber, its *percentage* of the total air has changed from 79.030 to 79.371 per cent. Consequently, to obtain the true percentage for the oxygen deficit, a common basis of comparison between the air entering and leaving the chamber must first be secured. This is done by finding a percentage value\* for the oxygen in the ingoing air which bears the same ratio to 79.371 that 20.940 does to 79.030. The equation will thus be  $79.030 : 20.940 :: 79.371 : x$ , in which  $x$  equals 21.030. By subtracting from this figure the percentage of oxygen in the air leaving the chamber as determined by analysis (19.595 per cent.), it is found that the oxygen deficit is 1.435 per cent. of the outgoing air. When the calculation is completed ( $99.571 \times 1.435$ ), 1.429 volumes is obtained as the amount of oxygen used, which is the actual value that was absorbed by the subject as stated in the original assumption.

Since the percentage of carbon dioxide in the ingoing air is so small, the calculation of the carbon-dioxide elimination may be carried out in a much simpler way. The difference between the percentage of carbon dioxide in the ingoing air (0.030) and that in the outgoing air (1.034) is 1.004 per cent. This, when multiplied by the volume of outgoing air, gives a volume of 1.000 for the carbon dioxide eliminated.

The respiratory quotient may be calculated from either the percentages or the volumes for the oxygen absorption and the

carbon-dioxide elimination  $\left\{ \frac{\text{CO}_2}{\text{O}_2} \right\}$ , giving a value of 0.700.

Table V presents these calculated results in tabular form.

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\* Ordinarily this is called a percentage value, but this cannot be strictly correct as the percentage composition of the gas would then be over 100 per cent. ( $79.371 + 21.031 = 100.402$ ). It is rather a volume relationship.

TABLE V

Tabular Presentation of Calculation of Gaseous Exchange from an Analysis of Chamber Air

	Ingoing Air		Outgoing Air	
	Volumes	%	Volumes	%
Carbon Dioxide.....	0.030	0.030	1.030	1.034
Oxygen.....	20.940	20.940	19.511	19.595
Nitrogen.....	79.030	79.030	79.030	79.371
Total.....	100.000	100.000	99.571	100.000

*Calculation:*  $79.030 : 20.940 :: 79.371 : x = 21.030$ .

$21.030 - 19.595 = 1.435$  per cent.  $O_2$  deficit.

$1.034 - 0.030 = 1.004$  per cent.  $CO_2$  increment.

$99.571 \times 1.004 = 1.000$  vols.  $CO_2$  eliminated.

$99.571 \times 1.435 = 1.429$  vols.  $O_2$  used.

$1.004 \div 1.435$  or  $1.000 \div 1.429 = 0.700$  R.Q.

In the experiments that are carried on with the respiration chamber at the New Hampshire Agricultural Experiment Station, the carbon-dioxide output is determined by weight and an analysis is made of a sample of the air leaving the chamber, so that the method of calculating the *amount* of oxygen used is different from that used in the illustrative calculation. For example, in a certain experiment the amount of carbon dioxide eliminated, as determined, was 78.54 grams, and an analysis of the outgoing air gave for carbon dioxide, 0.970 per cent., and for oxygen, 19.846 per cent. The percentage of nitrogen in the outgoing air, as calculated by difference, would thus be  $100.000 - (0.970 + 19.846) = 79.184$  per cent. Using the method previously outlined for calculating the oxygen percentage corresponding to the nitrogen percentage of 79.184, the equation would be  $79.030 : 20.940 :: 79.184 : x$ , with  $x$  equalling 20.981. The oxygen deficit therefore equals  $20.981 - 19.846$ , or 1.135 per cent. of the outgoing air. The carbon-dioxide increment equals  $0.970 - 0.030$ , or 0.940 per cent. The respiratory quotient would accordingly be  $0.940 \div 1.135$ , or 0.828. To compute the oxygen used by the animal, the 78.54 grams of carbon dioxide given off must first be converted to volume, that is,  $78.54 \times 0.5091$ , or 39.98 liters. This, divided by the respiratory quotient, gives 48.29 liters as the *volume* of oxygen used. When this volume of oxygen is



divided by the factor 0.7, 68.99 grams is obtained as the *weight* of oxygen used by the animal during the period.

### Summary

A gas-analysis apparatus based upon the Haldane principle has been devised for use especially with respiratory exchange apparatus in which a chamber is employed. It has a capacity of 40.04 cc. and readings can be made to 0.001 per cent. Samples of gases containing as much as 1.7 per cent. of carbon dioxide and from 78.4 to 79.6 per cent. of nitrogen can be analyzed with this apparatus. Analyses of outdoor air have given  $0.030 \pm 0.003$  per cent. for carbon dioxide and  $20.940 \pm 0.003$  per cent. for oxygen.

I desire to express my thanks to Mr. E. L. Fox, Miss Marion L. Baker, and Mrs. Lois A. Ritzman for their patience and painstaking care in making a large number of analyses in the development of this apparatus.

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# THE ACID-BASE EQUILIBRIUM IN DIABETIC COMA BEING A STUDY OF FIVE CASES TREATED WITH INSULIN \*\*

By

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This paper reports a study of the acid-base equilibrium in five cases of diabetic coma, discusses the value of the restricted use of bicarbonate of soda in combating acidosis, and describes a method by which the amount of acid in the body may be calculated in so far as present knowledge of the physiology of acid distribution in blood and other tissues permits.

Our interest in insulin for the present purpose lies chiefly in its use in the study of one of the most vital regulatory mechanisms known to physiology, the regulation of the reaction of the blood. Owing to the introduction of this preparation it is now possible to observe greater disturbances in acid-base equilibrium in the same individual than has been possible heretofore.

The data obtained in the study of four of the five cases are represented in a graphic way by means of "the CO<sub>2</sub> diagram" of Haggard and Henderson.<sup>1</sup> This diagram represents a moving picture of the changes through which the blood (and with the blood, the body in general) goes in recovery from acidosis, or in the development of acidosis. The interpretation of these diagrams may be made as readily as the clinician now interprets a temperature chart. The attempt is therefore made to relate the clinical and laboratory data so that the significance of the clinical findings may be better understood. If the meaning of the data is once grasped the clinician who is without laboratory facilities may outline with confidence the particular treatment indicated. The actual construction of the CO<sub>2</sub> diagram, the meaning of its several parts, and its interpretation in various disease states has been

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This paper is study No. 29 of a series of studies of the physiology and pathology of the blood from the Harvard Medical School and allied hospitals, the expenses of which have been defrayed in part by the Proctor Fund for the study of chronic disease.

\*The insulin used in the treatment of these patients was made by Eli Lilly & Co., under the trade name "Iletin" and was donated by them to the Boston clinicians, through whose courtesy we obtained it.

already discussed in previous papers from this clinic, to which the reader is referred.<sup>2, 3, 4</sup>

The recovery of five cases of diabetic coma is worthy of note. From January 1, 1912, to January 1, 1923, of a total of 844 cases of diabetes mellitus admitted to the Massachusetts General Hospital 68 died in coma and no case recovered from this state except one included in the present series.

It seems desirable to avoid any misunderstanding as to what our conception is of the clinical condition known as diabetic coma. The term "severe acidosis" is often used synonymously with that of coma, but the onset of the state of coma is usually the terminal condition of severe acidosis. There seems to be no agreement among clinicians or in accounts of the condition in the literature as to the definition of diabetic coma. If the patient can swallow liquids or can be aroused in any way he is thought by some not to be in coma; on the other hand, if the alveolar  $\text{CO}_2$  falls below 15 mm. he is considered by many to be in coma. All of our patients could swallow liquids after sufficient urging. They were unable to speak, but three of the five could respond when asked to show the tongue. Patient No. 4 could swallow liquids when the pH of his blood was 7.03 or thereabouts. The only instance cited in the literature with a reaction of the blood comparable to this was a case of Cullen's, quoted by Van Slyke,<sup>5</sup> having a pH of the blood of 6.95 just before death.

We believe that discussions of the precise definition of the actual state of coma are more or less irrelevant. It seems to us immaterial that one patient will eventually swallow as a result of highly adept attention on the part of a skilled nurse and that another patient does not swallow on perfunctory request to do so. In our opinion a diabetic patient who, without other cause, is generally oblivious to his environment, who is not roused at all or with great difficulty, and who is incapable of any complicated response, is in coma. In this clinical state the tension of alveolar  $\text{CO}_2$  is usually below 15 mm. of mercury and hyperpnea is invariably present.

#### METHODS

For details of technique used in obtaining arterial and venous blood and in determinations of  $\text{CO}_2$  and oxygen in blood, the reader is referred to the paper of Peters, Barr and Rule.<sup>6</sup> The following changes were made by us.

Sodium fluoride was added to the blood to prevent changes interfering with the absorption of  $\text{CO}_2$ , as suggested by Evans,<sup>7</sup> and the blood was kept on ice in the intervals between the determinations. The  $\text{CO}_2$  determinations were made with the constant volume apparatus of Van Slyke.<sup>8</sup> The oxygen determinations were made with a Van Slyke pipette having a stem graduated to 0.01 cc. and provided with a water-jacket. The oxygen was absorbed with pyrogalllic acid. For the determination of  $\text{CO}_2$  dissociation curves, the blood was placed in tonometers of 250 cc. capacity, containing various mixtures of  $\text{CO}_2$  and air and equilibrated in a water bath at  $37.5^\circ \text{C}$ . for ten minutes. The analysis of the blood for  $\text{CO}_2$  was made on a 1.0 cc. sample from the tonometer, and the gas in the tonometer was analyzed for  $\text{CO}_2$  in a Haldane apparatus after the blood had been removed for analysis.

The  $\text{CO}_2$  of the alveolar air was determined with the aid of a Haldane tube fitted with a sliding valve. We have found by experiment, the details of which will be published elsewhere, that samples of air taken at the end of normal expiration, rather than the average of samples taken at the end of both inspiration and expiration, agree closely with the tension of arterial  $\text{CO}_2$ , as shown by the A point on the  $\text{CO}_2$  dissociation curve determined on blood drawn as soon as possible after the alveolar samples were taken. The subject of alveolar air will be further considered below.

The general method of representation of the data in four cases is the same as that used by Means, Bock and Woodwell<sup>12</sup> and by Peters, Barr and Rule,<sup>6</sup> being a slight modification of the  $\text{CO}_2$  diagram of Haggard and Henderson mentioned above. The A point on this diagram is the point at which the  $\text{CO}_2$  content of the arterial blood falls on the  $\text{CO}_2$  dissociation curve. The tension of alveolar  $\text{CO}_2$  corresponds to the tension of the A point on the  $\text{CO}_2$  curve. The representation of the pH of the blood in the diagram is based on the fact that the hydrogen ion concentration of the blood may be calculated from the ratio  $\frac{\text{BHCO}_3}{\text{H}_2\text{CO}_3}$  as shown by L. J. Henderson.<sup>9</sup> Warburg<sup>10</sup> has recently noted that a correction should be made to the Henderson formula in order to get the exact pH of the blood, but since the total correction amounts to an increase of approximately pH 0.03, we have elected to use the more usual method of calculation.

The determinations of blood sugar and urinary nitrogen were made by the methods of Folin and Wu,<sup>11, 12</sup> of blood fat by the method of Bloor<sup>13</sup> and were carried out in the chemical laboratory of the hospital under the supervision of Dr. James L. Stoddard.

## CASES

*Case 1.* Hospital No. E. M. 253892. A girl of 12 years, admitted to the hospital for the first time March 13, 1922, with a history of increasing weakness, pain in back and legs, polyuria, nocturia, increased thirst and appetite, with loss of 15 pounds in one month. In the hospital she was shortly made sugar free and was discharged with a diet of C. 25, P. 30,



F. 60, and a blood sugar of 80 mgm. per 100 cc. of blood. Her weight was 29 kilograms.

After one month, glycosuria and all the former symptoms returned and she was readmitted May 9, 1922, with 4% of sugar in the urine and blood sugar of 232 mg. per 100 cc. of blood. Her diet could not be raised above C. 25, P. 35, F. 110. She was discharged on this diet with a blood sugar of 110 mg. per 100 cc. Her weight was 31.5 kilograms.

TABLE I

Date 1923	In- sulin Units	Diet			Sodium Bicar- bonate gm.	Diacetic Acid	Urine		Blood Sugar mg. per 100 cc.
		C	P	F			Nitrogen gm.	Sugar gm.	
Jan. 4 7:30 p. m. to 12 m.	50	54	0	0	6	-----	-----	27	7:30 p. m. 412 12:25 a. m. 28
Jan. 5	75	144	0	0	6	Lost	-----	-----	7:30 a. m. 310 4:00 p. m. 227
6	30	81	0	0	-----	0	26.45	30	10:00 a. m. 26 12:00 m. 286
7	30	40	40	0	-----	0	17.2	40	-----
8	10	20	20	0	-----	0	13.7	18	----- 167
9	0	10	10	0	-----	-----	20.8	51	----- 286
10	23	114	10	0	-----	-----	19.4	16	10:30 a. m. 286 3:30 p. m. -----
11	16	50	20	0	-----	0	2.32	23	----- 336
12	10	50	20	0	-----	-----	8.8	48	----- 288
13	10	10	10	0	-----	-----	-----	20	----- 223
14	10	28	10	0	-----	-----	-----	0	-----
15	---	10	10	0	-----	0	2.17	Trace	----- 220
16	---	20	10	0	-----	-----	2.24	0	-----
17	---	10	10	0	-----	-----	2.88	0	----- 160
18	---	20	10	0	-----	0	2.24	0	-----
19	---	20	20	0	-----	0	-----	0	----- 114
20	---	30	30	10	-----	-----	-----	0	-----
21	---	30	30	30	-----	0	3.52	0	-----
22	---	30	30	50	-----	0	2.89	0	----- 130
23	---	40	36	65	-----	0	3.88	0	-----
24	---	50	45	65	-----	0	3.75	0	-----
25	---	60	50	65	-----	0	-----	0	----- 184
26	---	60	50	65	-----	0	-----	Trace	-----
27	---	30	30	50	-----	0	-----	14.4	-----
28	---	30	30	50	-----	0	-----	16.0	-----
29	---	30	30	50	-----	0	6.76	Trace	----- 150
30	---	30	30	50	-----	0	-----	0	-----
Feb. 1	---	40	40	60	-----	0	-----	0	----- 92
2	---	40	45	65	-----	0	-----	0	-----
3	---	40	45	65	-----	0	-----	0	-----
4	---	40	45	65	-----	0	-----	0	-----
5	---	40	45	65	-----	0	-----	0	----- 139
6	---	40	45	65	-----	0	-----	0	-----
7	---	40	45	65	-----	0	-----	0	-----
8	---	40	45	65	-----	0	-----	0	----- 106
9	---	40	45	65	-----	0	-----	0	-----



Three days after discharge, she again had glycosuria and spent the following three months in a hospital for chronic disease. Two weeks after discharge from this hospital glycosuria again appeared, but she felt well and went to school until November 25. With the return of symptoms, she became more careless as to diet and during the Christmas holidays dietary restrictions were completely disregarded. Two days before admission on January 4 she became drowsy. The drowsiness increased to stupor from which she could be aroused with difficulty on the day of admission.

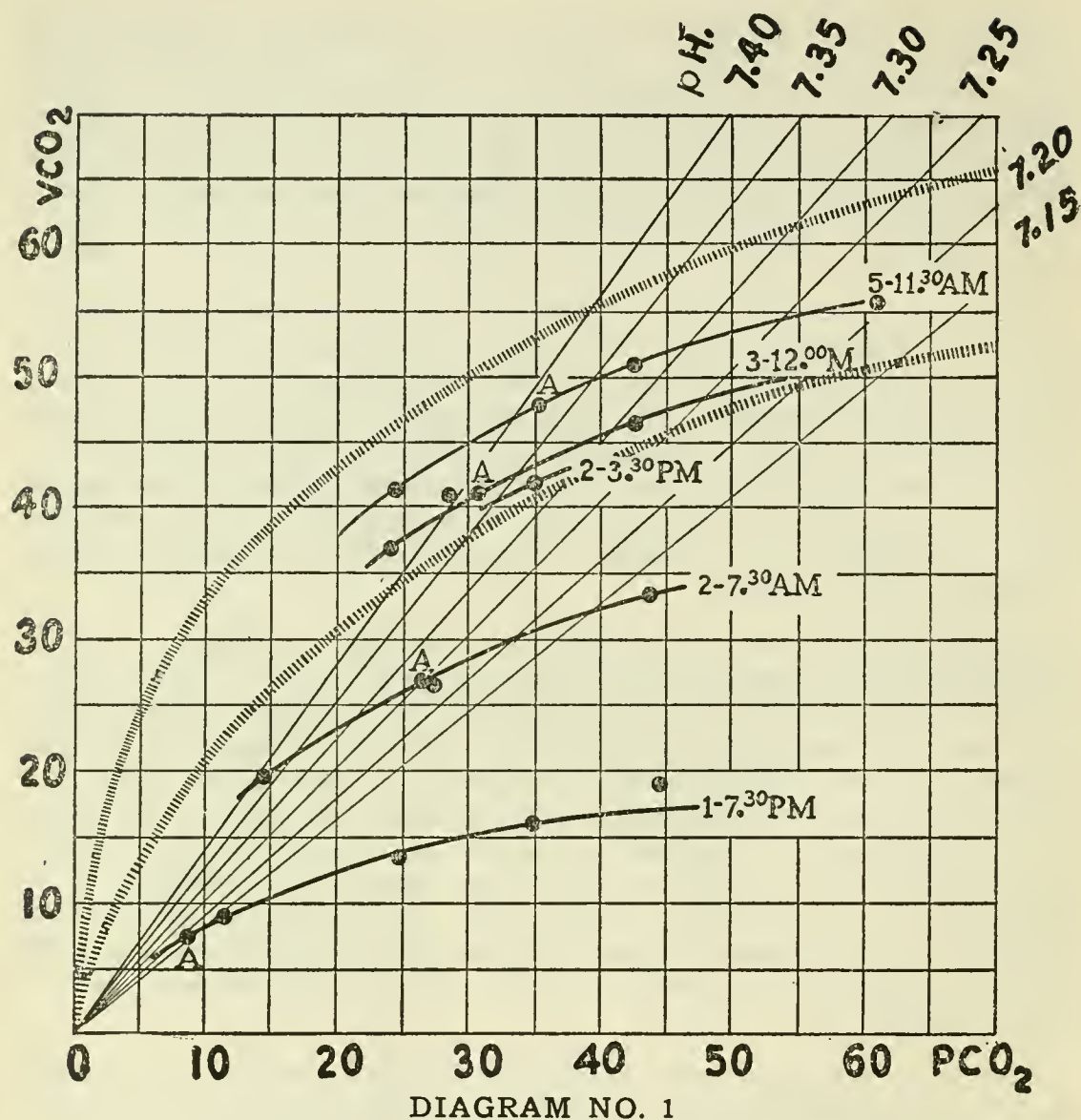
When first seen at 5:00 p. m. on January 4, 1923, she was in deep stupor, but could be induced to take fluids and made unsuccessful attempts to answer questions. She had deep sighing respirations, rate 30 to 35, pulse 130 and temperature 100.6°. The face was flushed, the lips bright red and there was a heavy odor of acetone on her breath. A supply of insulin was not available until 9:30 p. m. The total amount given is recorded in Table I, in which are recorded data with respect to subsequent diet, urinary findings, etc. Her weight on admission and at discharge from the hospital was 29 kilograms.

*Laboratory Data.* Arterial blood drawn as soon as possible after the admission of the patient on January 4 was found to have a CO<sub>2</sub> content of 7.5 volumes per cent. and the venous blood a CO<sub>2</sub> content of 8.7 volumes per cent. In Diagram No. 1, it will be seen that the A point on the CO<sub>2</sub> dissociation curve corresponds to a tension of 8.7 mm., which, from data of Krogh and others, closely approaches the tension of CO<sub>2</sub> in the alveolar air. The pH was 7.17. The level of the CO<sub>2</sub> curve at arterial CO<sub>2</sub> tension is only 15.7% of that on January 8, when her blood is assumed to be approximately normal. This change from the normal will indicate the severity of the acidosis present. An estimate of the amount of acid required to reduce the bicarbonate of the blood to this low level will be found on page 32.

Five hours after admission the venous CO<sub>2</sub> had risen to 17.6 volumes per cent., the alkali reserve was 23.3 volumes per cent., and we accordingly felt that the acidotic state was rapidly clearing up.

Twelve hours after insulin therapy was initiated, the arterial CO<sub>2</sub> had risen to 26.9 volumes per cent., giving an alveolar CO<sub>2</sub> tension of 26.5 mm. of mercury and a pH of 7.28. The bicarbonate of the blood at this point was 56% of her normal level and she was well out of her acidosis clinically.

On January 6, forty and one-half hours after the first observation, the arterial CO<sub>2</sub> had risen to 41.1 volumes per cent., the alveolar CO<sub>2</sub> tension to 30.7 mm., and the pH to 7.38. The bicarbonate level at this point was 86% of her normal amount. On January 8, sixty-four hours after admission, the arterial CO<sub>2</sub> was 47.8 volumes per cent., the alveolar CO<sub>2</sub> tension 35 mm. of mercury and the pH 7.38. A determination of alveolar CO<sub>2</sub> tension on January 24 gave a value of 34 mm., and we have no reason to doubt that the curve of January 8 is approximately at the normal level for this patient.



CO<sub>2</sub> Diagram No. 1. In this and in subsequent CO<sub>2</sub> Diagrams the ordinates represent volumes per cent. of CO<sub>2</sub>, the abscissae, the tension of CO<sub>2</sub> in mm. of mercury. The CO<sub>2</sub> dissociation curves are those of whole blood, completely oxidized, and are drawn free hand through the points as shown. The volumes per cent. of CO<sub>2</sub> contained in arterial blood corresponding to each curve are designated on the curve by the letter A. The tension of alveolar CO<sub>2</sub> may be read off directly, since it corresponds to the tension marked by an ordinate constructed through the A point. The zone marked by the heavy broken lines represents the level at which the CO<sub>2</sub> dissociation curves of normal individuals fall, as recorded in the literature. This zone is taken from the paper of Peters, Barr and Rule, to which reference is made in the text. The pH lines are constructed after the manner described by Van Slyke<sup>5</sup>. The individual curves are designated by time relationships: 1—7:30 A. M., being the morning of the first day in hospital, 2—7:30 A. M., the second day, etc.

The disappearance of acidosis under insulin therapy is striking. From January 4 to January 8 the total amount of insulin given was 195 units. There was given a total of only 12 grams of bicarbonate of soda, which may have had very little effect upon her acidosis. The rapid rise of  $\text{CO}_2$  in her blood probably indicates the rate at which the ketone bodies disappeared with the oxidation of sugar under the influence of insulin.

The high urinary nitrogen indicates the degree of body protein destruction. Although she did not attain nitrogen balance until approximately 16 days after admission, it is not unlikely that we could have secured this state much earlier had we had available a supply of insulin great enough to have kept up the injections in larger amounts than were given during the period of ten days following the recovery from coma.

From a comparison of data obtained on previous admissions of this patient to the hospital with the final tolerance now established, it is of interest that the accident of coma seems to have had no effect in reducing her tolerance for food.

The laboratory data relating to the blood are assembled in Table II.

TABLE II

Date, 1923	Arterial $\text{CO}_2$ Vol. %	Venous $\text{CO}_2$ Vol. %	Alkali Reserve Vol. %	Alveolar $\text{CO}_2$ mm. of Mercury	pH	CO <sub>2</sub> Dissociation Curve	
						Tension mm.	Vol. %
Jan. 4 7:30 p.m.	7.5	8.5	-----	8.7	7.17	11.6 24.8 34.8 44.6	8.9 13.55 16.0 19.0
5 12:25 a.m.	-----	17.6	23.2	-----	-----	-----	-----
5 7:30 a.m.	26.9	30.3	-----	26.5	7.25	14.6 27.4 43.8	19.6 26.7 33.5
5 3:30 p.m.	-----	42.7	53.9	-----	-----	35.0	41.9
6 12:00 n.	41.1	44.2	-----	30.7	7.38	24.1 28.6 42.6	37.0 41.0 46.4
8 11:30 a.m.	47.8	49.9	59.2	35.2	7.38	24.6 42.7 61.0	41.4 51.0 55.8
10 10:30 a.m.	-----	29.1	39.1	-----	-----	-----	-----
10 3:30 p.m.	34.6	37.2	-----	-----	-----	-----	-----
11 11:45 a.m.	-----	48.2	57.7	-----	-----	-----	-----
13	-----	-----	64.6	-----	-----	-----	-----
16	-----	-----	64.6	-----	-----	-----	-----



**Case 2.** Hospital No. E. M. 254069. This was a boy of fourteen and a half years, who had always been well and from whom no symptoms could be elicited until one week before admission to the hospital, when he complained that his legs were weak and he felt tired on exertion. He then noticed a greatly increased thirst and appetite, "was drinking all of the time," "used to wake up at night to drink," "ate enough for ten boys." The weakness of his legs increased so that for four days before entrance he did not go to school, but remained in bed much of the time. Thirty-six hours before admission he began to vomit, and through the night and following day vomited everything taken. During the day before admission he became drowsy and by the late evening was in deep stupor.

He was first seen at 4:30 a. m. on January 15, 1923, when he was found in coma so deep that he could not be aroused by any of the usual stimuli. His hyperpnea was of extreme degree and the odor of acetone filled the room. The skin had an ashy pallor, the lips were pale, the eyeballs soft, the pulse was just perceptible, rate 150, and the temperature was 96°. When a needle was inserted into the median basilic vein, blood would flow only when the arm was stroked, a small specimen only being obtained for blood sugar. He was immediately given an intravenous injection of 300 cc. of 5% glucose and a subpectoral injection of 1800 cc. containing 800 cc. of 5% glucose. He was also given a rectal injection containing 6 grams of bicarbonate of soda, of which a portion was not retained. No supply of insulin was available until 9:30 a. m., when the treatment with this preparation was begun. He had improved sufficiently by this time to be able to swallow, but there was no other clinical evidence of change. When the first injection of insulin was given, he had a small, scarcely palpable pulse of 150 to 160 and his blood pressure could not be satisfactorily registered. The amount of insulin given, the diet, urinary findings, etc., are recorded in Table III.

Analysis of the data in Table III, for the fifth day, shows that he had about 56 grams of carbohydrate available from all sources, diet and body protein, and of this he excreted 44 grams. It appears probable that the ten units of insulin, which were given him that day, were responsible for the utilization of most of the 12 grams of carbohydrate burned. On the sixth day, without insulin, he utilized approximately 25 grams of carbohydrate and from this day his tolerance rapidly increased. On the fourteenth day, he was on a diet of C. 60, P. 60, F. 70, with a blood sugar of 95 mg., and by the twenty-eight day he was able to take a diet of C. 180, P. 60, F. 120, representing 227 grams of carbohydrate, without an increase in his blood sugar above 95 mg. He was discharged on a diet of C. 140, P. 60, F. 120. His least weight was 38 kilograms, which increased to 40 kilograms by the time of discharge.

*Laboratory Data.* At 9:00 a. m. on January 15, the arterial CO<sub>2</sub> was found to be 11.2 volumes per cent., the venous CO<sub>2</sub> (with tourniquet applied) 19.1 volumes per cent., the alveolar CO<sub>2</sub> tension 12.5 mm. of mercury and the pH of the arterial blood 7.20.

Twenty-four hours after the first administration of insulin, there was very little clinical evidence of acidosis. The CO<sub>2</sub> content of the arterial



TABLE III

Date 1923	In- sulin Units	Diet			Sodium Bicar- bonate gm.	Diacetic Acid	Urine		Blood Sugar mg. per 100 cc.	
		C	P	F			Nitrogen gm.	Sugar gm.		
Jan. 15										
9:30 a. m.	90	200	0	0	19	+	9.9	154.0	9:30 a. m.	454
to 12 m.						+			4:45 p. m.	444
Jan. 16	45	99	0	0	-----	+	18.0	63.8		290
17	20	58	20	0	-----	+	20.0	73.5		
18	15	40	20	0	-----	0	10.9	28.5		200
19	10	10	20	0	-----	+	12.8	44.0		222
20	----	10	10	0	-----	+	11.0	25.5		175
21	----	10	10	0	-----	+	11.1	19.0		
22	----	30	30	50	-----	Trace	11.8	Trace		155
23	----	30	30	50	-----	0	15.2	0		152
24	----	30	30	50	-----	0	-----	0		
25	----	40	50	60	-----	0	-----	0		108
26	----	40	50	60	-----	0	-----	0		
27	----	40	50	60	-----	0	-----	0		
28	----	60	60	70	-----	0	8.48	0		
29	----	60	60	70	-----	0	-----	0		95
30	----	60	60	70	-----	0	-----	0		
31	----	70	70	110	-----	0	-----	0		
Feb. 1	----	70	70	110	-----	0	-----	0		93
2	----	70	70	120	-----	0	-----	0		
3	----	70	70	120	-----	0	-----	0		
4	----	80	60	120	-----	0	-----	0		
5	----	100	60	120	-----	0	-----	0		84
6	----	110	60	120	-----	0	-----	0		
7	----	120	60	120	-----	0	-----	0		
8	----	120	60	120	-----	0	-----	0		85
9	----	140	60	120	-----	0	-----	0		
10	----	160	60	120	-----	0	-----	-----		
11	----	180	60	120	-----	0	-----	-----		
12	----	140	60	120	-----	0	-----	-----		95

blood was then 39.5 volumes per cent., the pH 7.38, the venous CO<sub>2</sub> content 45.1 volumes per cent. and the alveolar CO<sub>2</sub> tension 29.4 mm. This striking change is well shown in Diagram No. 2.

On January 17, two days after admission, there was a slight downward shift in the level of the blood bicarbonate, which, however, on the following day approximated his normal level. The administration of 155 units of insulin had resulted in clearing up the acidosis completely.

It seems clear from the amount of sugar eliminated during the first three days that too much carbohydrate was given during this interval. Whether or not this was injurious to the patient is a debatable question, but it served as insurance against a hypoglycemic reaction, and until more knowledge is obtained as to the effects of flooding the organism with sugar as well as to the oxidizing power of insulin, it seems to us advisable to have avail-

able in the body an excess of carbohydrate during the period when insulin is being pushed vigorously.

The rapid fall of a somewhat high urinary nitrogen is satisfactory. Although a positive nitrogen balance was not obtained until approximately ten days after admission, the total weight

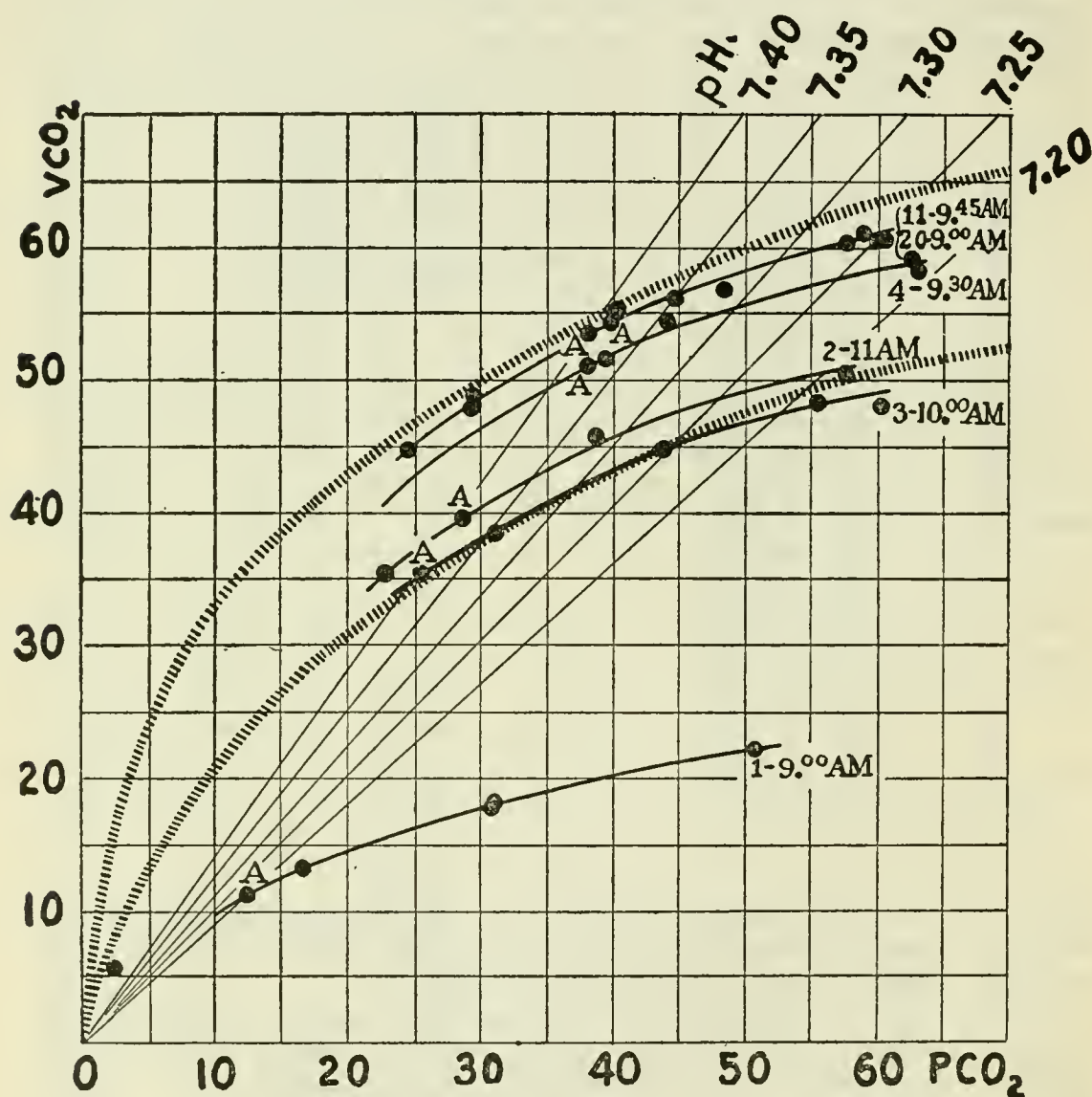


DIAGRAM NO. 2

CO<sub>2</sub> Diagram No. 2.

loss was not great and could possibly have been avoided by the continuance for several days of a high calory diet together with insulin.

The concentration of the blood in this patient was greater than we have encountered in any other condition. The oxygen capa-

city of the blood on entrance was 26.0 volumes per cent.; on the following day it was 24.6 volumes per cent.; and on the third day it was 19.2 volumes per cent. If simple dehydration could account for this concentration one would expect that dilution of the blood would occur within twenty-four hours on an adequate fluid intake such as this patient had. Concentration of the blood of less degree in diabetic patients has often been observed to disappear within twenty-four hours on a high fluid intake. It is possible that the failure of the present case to dilute his blood within the usual period was associated with the very high sugar intake and output during that time.

The laboratory data relating to the blood are shown in Table IV.

TABLE IV

Date 1923	Arterial CO <sub>2</sub> Vol. %	Venous CO <sub>2</sub> Vol. %	Alkali Reserve Vol. %	Alveolar CO <sub>2</sub> mm. of Mercury		pH	CO <sub>2</sub> Dissocia- tion Curve		Oxygen Capac- ity Vol. %
				A mm.	a mm.		Ten- sion mm.	Vol. %	
Jan. 15 9:00 a. m.	11.2	19.1	-----	12.5	-----	7.20	16.6 31.0 31.2 50.6	13.3 17.9 18.2 22.2	26.0
15 4:45 p. m.	-----	34	-----	-----	-----	-----	-----	-----	-----
16 9:00 a. m.	39.5	45.1	-----	29.4	-----	7.38	22.8 38.6 57.6	35.3 45.9 50.6	24.6
17 11:00 a. m.	-----	-----	-----	-----	31.5	-----	-----	-----	-----
17 10:00 a. m.	35.3	38.5	-----	26	27.2	7.39	25.5 31.1 43.7 55.4 60.2	35.2 38.5 44.8 48.4 48.2	19.2
18 9:30 a. m.	51.0	53.4	-----	37.8	37	7.38	24.5 39.4 62.4 63.0	44.8 51.7 59.4 58.4	-----
26 9:45 a. m.	54.3	58.2	-----	39.8	40.7	7.37	26.0 44.0 45.2 57.7 59.0 60.6	45.6 54.4 55.2 60.5 61.1 61.0	20.0
Feb. 6 9:00 a. m.	53.5	59.8	-----	38.0	40.7	7.38	29.4 29.3 44.7 48.4 70.3	48.7 48.0 56.0 56.8 60.9	19.6

Case 3. Hospital No. W. M. 250960. This patient, a married woman of twenty-six years, had nothing of note in her past or family history. Two years ago she had a sudden increase in appetite and gained from 108 to 128 pounds in one month. A month later she began to lose weight in spite of a good appetite, and two months after this developed weakness,



excessive thirst and polyuria. After three or four weeks, the symptoms increased to such an extent that a physician was consulted and a diagnosis of diabetes mellitus made.

Following this, she was on a well regulated diet, kept sugar free, and felt very well until last summer, when, owing to difficulties about gauging amounts of food properly, glycosuria developed at times. About two months before admission, she began to disregard her diet more consistently, first taking extra amounts of allowed foods, then small amounts of high carbohydrate foods, until at Christmas time she broke diet entirely.

Two weeks before admission, she began to feel very tired and nervous. Her stomach felt "uneasy" much of the time and she had excessive thirst and polyuria. The day before admission, realizing that she was getting into difficulty, she decided to resume her diet and started a complete fast. That morning she complained of feeling cold, felt much weaker and was drowsy. Her mother noticed rapid breathing and a sweet odor on her breath. She became worse during the night and at times was unable to recognize members of her family. In the morning, she was brought to the hospital.

When seen at 11:30 a. m., she was in deep stupor, but could be aroused to take fluids and attempted to answer questions. She had hyperpnea of moderate degree, a thready pulse of 130 and temperature of 96.6°. There was a strong odor of acetone. She was given insulin at the rate of 10 units an hour, orange juice 3 ounces an hour, and as much of other fluids as could be forced. The data of interest are assembled in Table V.

Analysis of the data of the fourth day shows that she already had a small sugar tolerance, as she excreted only 23 grams of a total of about 30 grams of available glucose. On the ninth day, she excreted 11.4 grams of sugar from a total of about 35 grams. When the diet had been increased to C. 20, P. 35, F. 100, she exhibited a slowly rising blood sugar and then small amounts of urine sugar. In spite of this, 10 grams of fat were added to her diet. Two days later she was sugar free, but her blood sugar was practically unchanged. On this day, she burned practically all of the 53 grams of carbohydrate derived from her diet plus 10 grams derived from katabolism of body protein, having not yet attained nitrogen equilibrium. At this time, the use of insulin was resumed. On a diet of C. 40, P. 45, F. 130 and 18 units of insulin, she attained nitrogen equilibrium, was sugar free and had a diminishing blood sugar. At discharge, on this diet and 15 units of insulin daily, she had no glycosuria, although her blood sugar remained at a high level. Her weight on January 10 was 42 kilograms; on January 22, it was 40; and on February 12, it was 41.5 kilograms.

*Laboratory Data.* On the first examination of the blood in this patient, drawn at once after the first injection of insulin on January 9, the arterial CO<sub>2</sub> was found to be 7.1 volumes per cent., the venous CO<sub>2</sub> 9.4 volumes per cent., the alkali reserve 15.9 volumes per cent., the alveolar CO<sub>2</sub> tension 8.3 mm. of mercury and the pH of the arterial blood 7.17. The bicarbonate of the blood at the arterial CO<sub>2</sub> tension of 8.3 mm. was



TABLE V

Date 1923	In- sulin Units	Diet			Sodium Bicar- bonate gm.	Diacetic Acid	Urine		Blood Sugar mg. per 100 cc.
		C	P	F			Nitrogen gm.	Sugar gm.	
Jan. 9 11:30 a.m. to 12 m .	80	110	0	0	9	+ +	3.8	30	11:30 a. m. 334 1:40 p. m. 400 5:45 p. m. 336 11:00 p. m. 208
Jan. 10	75	75	50	0	-----	0	-----	15	8:30 a. m. 296 4:45 p. m. 286
11	30	25	50	0	-----	Trace	5.8	0	9:15 a. m. 258 3:30 p. m. 151
12	0	5	12	0	-----	+ +	6.9	23.3	-----
13	10	30	20	0	-----	+ + +	5.5	14.5	-----
14	5	35	20	0	-----	+	7.6	26.4	-----
15	5	30	20	0	-----	+	5.6	17.3	268
16	----	20	20	0	-----	+	7.8	25.4	245
17	----	10	10	0	-----	+	6.8	11.4	228
18	----	10	10	0	-----	+	5.0	0	220
19	----	10	10	0	-----	+	5.7	0	224
20	----	10	10	30	-----	+	3.9	0	180
21	----	10	20	30	-----	+	2.9	0	-----
22	----	15	20	50	-----	+	3.9	0	183
23	----	20	25	60	-----	+	4.4	0	212
24	----	20	30	70	-----	0	5.9	0	-----
25	----	20	30	70	-----	+	3.8	0	188
26	----	20	30	70	-----	0	6.34	0	200
27	----	20	30	70	-----	Trace	5.1	0	183
28	----	20	30	80	-----	"	4.2	0	-----
29	----	20	30	90	-----	0	-----	0	190
30	----	20	30	100	-----	+	4.6	0	209
31	----	20	35	100	-----	+	3.9	0	224
Feb. 1	----	20	35	100	-----	+	5.8	0	204
2	----	20	35	100	-----	+	6.2	Trace	222
3	----	20	35	100	-----	+	6.6	"	250
4	----	20	35	100	-----	+	6.8	17.5	-----
5	----	20	35	130	-----	+	5.6	14.2	-----
6	----	20	35	130	-----	+	6.3	0	250
7	10	40	35	130	-----	Trace	6.3	0	7:45 a. m. 258 11:50 a. m. 201 4:45 p. m. 168 8:15 p. m. 135
8	10	40	35	130	-----	+	4.9	0	7:45 a. m. 222 11:00 a. m. 204 4:45 p. m. 180 8:30 p. m. 141
9	10	40	35	130	-----	Trace	3.9	0	190
10	24	40	35	130	-----	0	6.58	0	208
11	24	40	35	130	-----	-----	4.6	-----	-----
12	24	40	35	130	-----	-----	5.5	-----	198
13	15	40	45	130	-----	-----	5.9	-----	258
14	18	40	45	130	-----	-----	5.9	-----	244

15.5% of her normal base as determined from the data of January 25. This amount of reduction in base is the same as was found in Case 1. At 5:00 p. m., the venous  $\text{CO}_2$  content had risen to 15.6 volumes per cent., the alkali reserve to 23.9 volumes per cent., and by 11:00 p. m., eleven hours after admission, the venous  $\text{CO}_2$  was 24 volumes per cent.

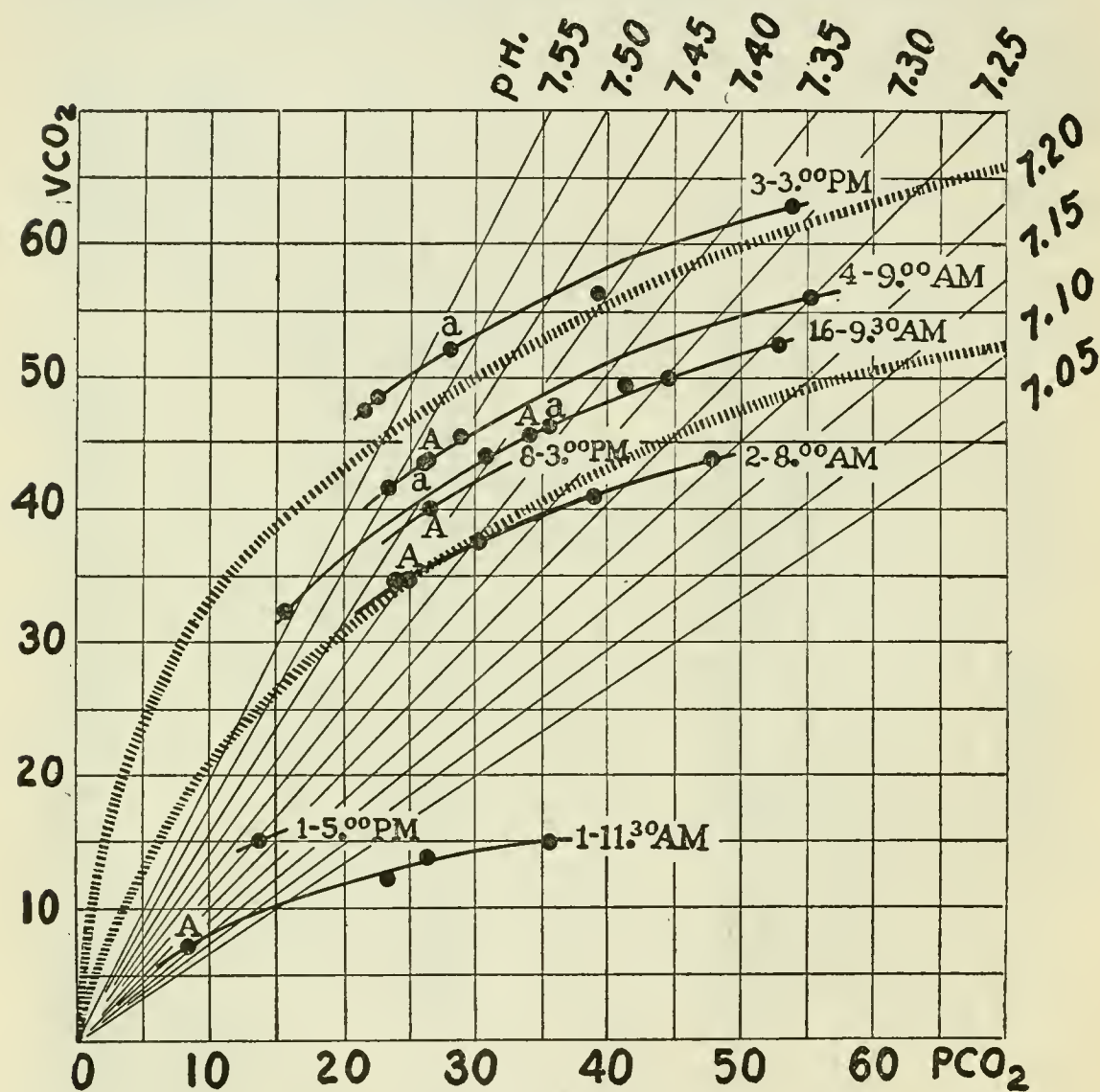


DIAGRAM NO. 3

$\text{CO}_2$  Diagram No. 3. The points marked "a" designate  $\text{CO}_2$  contents of arterial blood, as determined by the tension of alveolar  $\text{CO}_2$  found by the Haldane method.

At 8:00 a. m. on January 10, twenty hours after the first injection of insulin, the arterial  $\text{CO}_2$  was 34.7 volumes per cent., the venous  $\text{CO}_2$  35.8 volumes per cent., the alveolar  $\text{CO}_2$  tension 25 mm. of mercury and the pH of the arterial blood 7.39. There was no clinical evidence of acidosis, the patient felt comfortable and seemed remarkably well. There had been given 110 units of insulin. A total of 9 grams of bicarbonate of soda had been administered.

On January 11, after having had a total of 175 units of insulin, a remarkable condition was found. The level of the  $\text{CO}_2$  dissociation curve proved to be above the limits recognized as normal. The alveolar  $\text{CO}_2$  tension, determined with the aid of the Haldane tube, at this time was 28 mm. of mercury, which corresponds to an arterial  $\text{CO}_2$  content of 52 volumes per cent. (determined by the point at which the 28 mm. ordinate crosses the  $\text{CO}_2$  dissociation curve). The pH of the arterial blood at this time was 7.57. We failed to determine the reaction of the urine, but presumed we were dealing with an alkalosis.

Our explanation for this phenomenon, observed also to a less degree in Case 4, is a wholly tentative one. It may be assumed that the burning of ketone bodies had gone on rapidly to completion, freeing rather quickly all the base combined with acid in the body. To account for the very high level of bicarbonate we may also assume that an extraordinary amount of base had been called out from fixed stores of alkali, such as from the bones, and that when the acute need for base in the neutralization of acid had been taken away the ability of the organism again to fix base or to excrete it via the kidneys had lagged enough to produce an alkalosis.

A change in the threshold of the respiratory center must also be assumed to account for the condition found. Normally this center would have attempted to compensate for the increased blood alkali and have maintained a normal or nearly normal pH by diminishing respiratory exchange with a consequent increase in the  $\text{CO}_2$  content of the blood and alveolar air. In the present case the opposite condition of hyperventilation seems to have persisted, washing out  $\text{CO}_2$ , so as to give the comparatively low tension of 28 mm. in the alveolar air. It is as if the respiratory mechanism had been set at a high pace during the period of acidosis and had continued overventilation of the lungs after the acidosis no longer existed.

On January 12, eighteen and one-half hours after the last observation, it will be noted in Diagram No. 3 that a very appreciable fall in the level of blood bicarbonate had occurred, the arterial  $\text{CO}_2$  having dropped from 53 volumes per cent. to 43.7 volumes per cent. and the pH, although still above the normal range, had swung back to 7.47. In other words, in this period of time a readjustment of considerable extent had occurred in the distribution of alkali. Either a redistribution of base in the body or elimination of base through the kidneys or both had occurred.



The data of January 25 show a perfectly normal relationship in all respects.

In this patient the urinary nitrogen fails to show any increase above the normal protein katabolism. The nitrogen balance in this patient appears not to have been disturbed.

The data discussed above are to be found in Table VI.

TABLE VI

Date 1923	Arterial CO <sub>2</sub> Vol. %	Venous CO <sub>2</sub> Vol. %	Alkali Reserve Vol. %	Alveolar CO <sub>2</sub>		pH	CO <sub>2</sub> Dissocia- tion Curve		Oxygen Capac- ity Vol. %
				A mm.	a mm.		Ten- sion mm.	Vol. %	
Jan. 9 11:30 a. m.	7.1	9.4	15.9	8.3	-----	7.17	8.7 23.4 26.3 35.7	7.2 12.3 13.8 14.9	22.5
9 5:00 p. m.	-----	15.6	23.9	-----	-----	-----	13.7	15	-----
10 11:00 p. m.	-----	24.0	-----	-----	-----	-----	-----	-----	-----
10 8:00 a. m.	34.7	35.8	-----	25.0	-----	7.39	24.1 30.4 39.2 47.7	34.3 37.6 41.0 43.8	-----
11 4:30 p. m.	-----	41.8	-----	-----	-----	-----	-----	-----	-----
11 3:30 p. m.	-----	-----	-----	-----	28	7.57	21.6 22.6 39.3 53.8	47.5 48.4 56.4 62.9	-----
12 9:00 a. m.	44.0	-----	-----	26.4	26.2	7.47	-----	-----	-----
16 3:00 p. m.	40.1	41.7	-----	-----	26.5	7.44	-----	-----	-----
25 2:30 p. m.	45.6	52.4	-----	34.2	35.7	7.38	15.7 30.8 44.6 53.0	32.3 44.0 50.0 52.5	18.3

**Case 4.** Hospital No. E. M. 254343. This patient, a boy of fourteen, had a history of onset of polydipsia, polyuria and loss of weight one year ago. By June 1922, loss of weight and appearance of ill health resulted in a consultation with the family physician, who made a diagnosis of diabetes and prescribed a diet without potatoes, desserts or candy. Bread and all other foods were permitted in unrestricted amounts. In August, 1922, he entered a suburban hospital, where he remained for five weeks on a dietetic regime not carefully regulated. He never became sugar free and his symptoms were only moderately relieved. Upon returning home, he led a reasonably active life until two weeks before admission to the Massachusetts General Hospital on January 29. During this time, he was easily fatigued and spent most of his time lying on a couch at home. He slept a great deal and suffered from excessive thirst, polyuria and loss of appetite. He was frequently nauseated and occasionally vomited. Two days before admission, he was quite drowsy and slept most of the day. The next day he seemed better and was up and about, but on the day of admission the drowsiness increased until by afternoon he was in deep stupor.



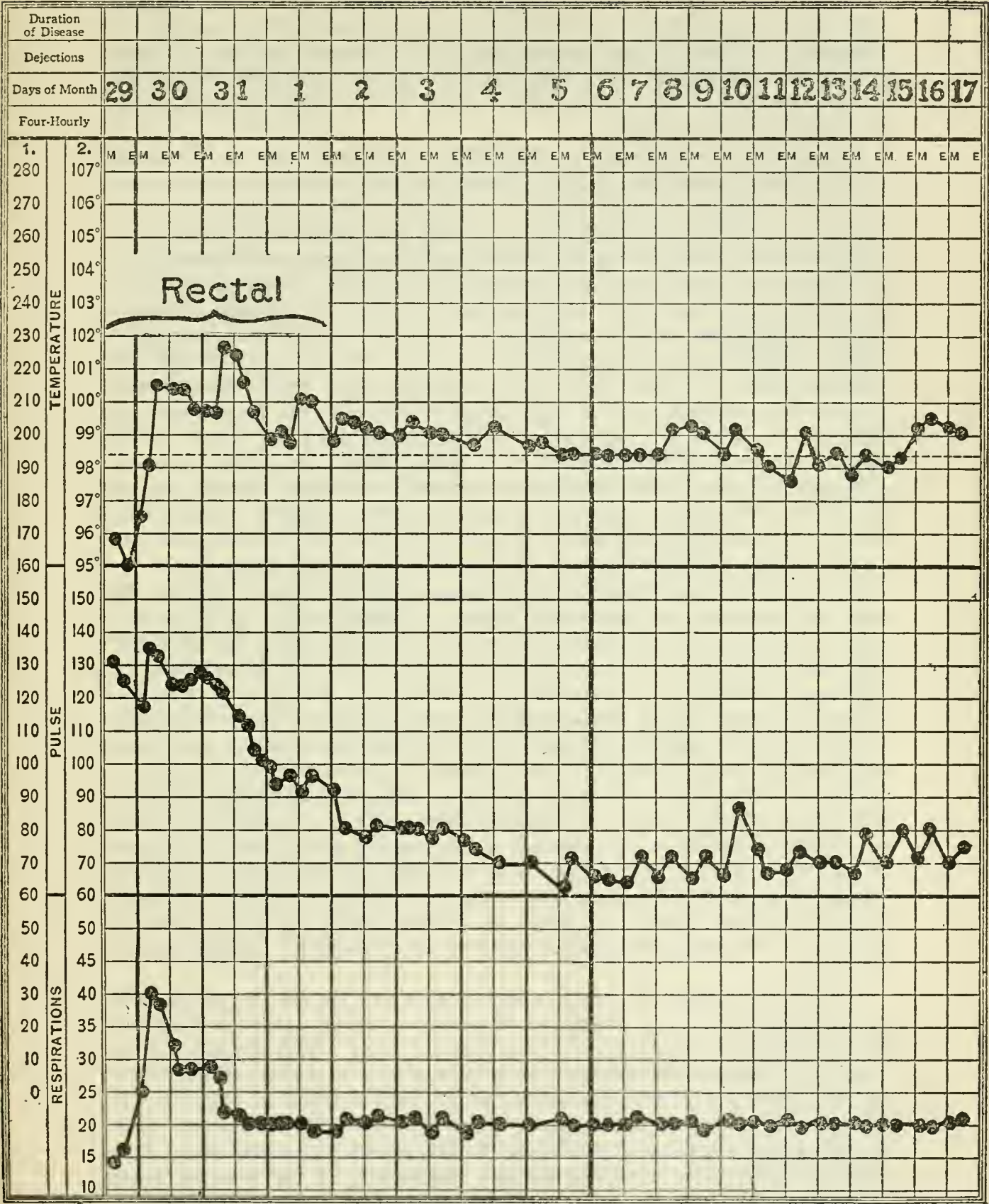


CHART NO. 1

When seen at 7:20 p. m., he could not be aroused, but would swallow when fluids were poured into his mouth. He seemed almost completely anesthetic. He had a rectal temperature of  $95^{\circ}$ , a barely perceptible pulse of 130, and respirations of 15, which were of maximal capacity and audible all over the ward. The cheeks were flushed, the lips bright red, the skin dry, scaling and very cold.

In addition to warmth and a subpectoral infusion of 2000 cc. of normal saline, he was given immediately 15 units of insulin intramuscularly and this was continued in this amount every hour. He was given about 4 ounces of orange juice every hour and other fluid by mouth. Up to midnight, he had been given 60 units of insulin, 105 grams of carbohydrate and 3000 cc. of fluid. The only change noticed in his condition was that his breath was less heavy with acetone and that he refused fluids by mouth. The blood  $\text{CO}_2$ , as mentioned below, not having changed appreciably, alkali therapy was instituted, 4 grams of bicarbonate of soda in a rectal tap of 6 ounces of 5% glucose being given every three hours. Insulin was reduced to 10 units an hour. At 6:45 a. m., he showed the first clinical signs of improvement. His temperature had come up to normal, his respirations changed to a rapid, quiet type, 40 per minute, although still quite deep. His pulse remained rapid and thready and, as in all the other cases, returned to normal slowly, lagging considerable behind improvement in all other respects. The chart of the case is reproduced as typical of the reaction of a patient recovering from coma.

During all of the second day he remained drowsy, slept much of the time and presented a remarkable degree of hyperesthesia of his entire body. Improvement continued until by the end of the third day his blood  $\text{CO}_2$  had reached a low normal level. On the fourth day, bicarbonate of soda was omitted and on the fifth day there was a return of acidosis, shown clinically and by blood findings. Alkali was again given by mouth at the rate of 3 grams an hour until 21 grams was given, which was found sufficient to raise his blood  $\text{CO}_2$  to a nearly normal level. Again, after a period of fifteen hours without alkali, there was a fall of about 7 volumes per cent. in the level of his  $\text{CO}_2$  dissociation curve at a tension of 40 mm. of mercury. Twice he had attained a low normal blood alkali level and twice, when sodium bicarbonate was omitted, without other significant change in his treatment, there was a considerable drop in the blood alkali. During this time, there was never more than a trace of ferric chloride reaction in the urine. Mention is made below of the urine findings at this time.

A sufficient supply of insulin was available and its use, to the amount of 15 units a day, was continued in order to avoid undernutrition and because of his known very low tolerance for food. With the aid of this amount of insulin, he was able to utilize a diet of C. 40, P. 40, F. 75 on the twelfth day with the excretion of only a trace of sugar and a gradually diminishing blood sugar. Then with 20 units of insulin daily, he was given a diet of C. 40, P. 50, F. 125, on which he was sugar free and his blood sugar remained normal. Inspection of the data of Table VII, from this time on, will reveal the method of dietary regulation in



an effort to find the maximum food allowance that could be taken with 20 units of insulin and the maintenance of a normal blood sugar. His weight on admission was 39 kilograms and remained at that figure. The data, with reference to amount of insulin, diet, urinary findings, etc., are shown in Table VII.

TABLE VII

Date 1923	In- sulin Units	Diet			Sodium Bicar- bonate gm.	Diacetic Acid	Urine		Blood Sugar mg. per 100 cc.
		C	P	F			Nitrogen gm.	Sugar gm.	
Jan. 29 7:30 p. m. to 12 m.	60	105	0	0	-----	+++	-----	-----	7:40 p. m. 454 11:30 p. m. 454
Jan. 30	210	93.5	0	0	24	++	3.4	8.8	6:45 a. m. 420 3:00 p. m. 296
31	95	72	0	0	12	0	2.3	Trace	108
Feb. 1	35	64	40	0	4	0	3.12	0	177
2	35	40	40	50	21	0	2.8	0	408
3	15	40	40	50	27	0	2.7	0	364
4	15	40	40	50	22	0	5.3	0	-----
5	15	40	40	50	-----	0	6.5	5.2	286
6	15	40	40	50	-----	0	8.35	8.0	-----
7	15	40	40	75	-----	0	7.70	Trace	-----
8	15	40	40	75	-----	0	-----	24.0	266
9	15	40	40	75	-----	0	10.4	Trace	-----
10	20	40	40	100	-----	0	-----	-----	222
11	20	40	50	125	-----	0	7.9	0	-----
12	20	40	50	125	-----	0	6.8	0	169
13	20	40	50	125	-----	0	4.8	0	162
14	20	40	50	125	-----	0	-----	0	-----
15	20	40	50	125	-----	0	5.6	0	148
16	20	40	50	125	-----	0	3.5	0	-----
17	20	40	50	125	-----	0	5.5	0	-----
18	20	40	50	125	-----	0	5.1	0	-----
19	20	40	60	125	-----	0	3.5	0	126
20	20	40	60	125	-----	0	-----	0	-----
21	20	40	60	125	-----	0	-----	0	-----
22	20	40	60	125	-----	0	6.1	0	-----
23	20	40	60	125	-----	0	-----	-----	129
24	20	45	60	125	-----	0	-----	-----	-----
25	20	45	60	125	-----	0	-----	-----	-----
26	20	50	60	125	-----	0	-----	-----	-----
27	20	55	60	125	-----	0	-----	-----	130
28	20	60	60	125	-----	-----	-----	-----	-----

*Laboratory Data.* A specimen of arterial blood, obtained twenty minutes after the first injection of insulin, contained 6.2 volumes per cent. of CO<sub>2</sub>, and the pH was 7.03. The venous CO<sub>2</sub> content was 9.9 volumes per cent., the alkali reserve 13.8 volumes per cent., and the alveolar CO<sub>2</sub> tension 9.7 mm. of mercury. These figures indicate that the blood must have lost more than 90% of its available base. Four hours later, after 60 units of insulin had been given and notwithstanding the fact that considerable quantities of ketones must have disappeared, very little change



with respect to  $\text{CO}_2$  in the blood was found. A possible explanation of this phenomenon was that some other acid than the ketone group was responsible for the persistence of acidosis, and it was therefore decided to give alkali to take up the extra acid. After alkali therapy was begun, the response was slow, as inspection of diagram No. 4 will show, but it was satisfactory and we believe that the use of alkali in this patient was life-saving. As noted above, on two occasions, there was a return of acidosis following the omission of soda, although the absence of ketones by the ferric chloride test was continuous and complete. On the occasion of the first relapse into acidosis on the fifth day and for several subsequent days, the titration of the urinary acidity, after the technique of Van Slyke and Palmer,<sup>14</sup> gave the following results, the figures as given being for grams per liter:

TABLE VIII.

Date	Creatin and Creatinin	Total Organic Acid .1 N	Total* Acetone .1 N
Feb. 2	.546	2610	43
Feb. 3	.558	960	0
Feb. 4	.585	380	109
Feb. 5	.640	190	35
Feb. 6	.541	200	10

The nature of the organic acid remains unknown. A review of the cases of diabetes dying in coma at the Massachusetts General Hospital from January 1, 1912, to January 1, 1923, has disclosed the fact that 15 of a total of 68 died with either a very slight ferric chloride test for diacetic acid in the urine or none at all. A number of them had a high output of ammonia. It seems not unlikely that certain of these cases may belong to the same group as does the case under discussion here. The cases reported by Rosenbloom,<sup>15</sup> McCaskey<sup>16</sup> and Starr and Fitz<sup>17</sup> are possibly in the same category. Apart from its merit as a therapeutic agent the value of insulin must be emphasized in this connection. It enabled us to recognize at once the existence of a type of acidosis that must be considered in every case of severe diabetic acidosis.

The significance of lipemia in diabetes is unknown, but in this case it may be related to the other disturbances noted with respect to acid formation.

The  $\text{CO}_2$  curves and alveolar  $\text{CO}_2$  determinations of February 5 and 8 indicate a pH of the blood of 7.49, more alkaline than normal. This may have been associated with the alkali given, or it may be explained on the same general basis as was the similar con-

\* These analysis were made for us at the Peter Bent Brigham Hospital by Drs. R. Fitz and P. Starr.

dition in Case 3—freeing of base from acid with a resulting temporary alkalosis, not associated with symptoms in either case. For other details of blood data reference should be made to Table IX.

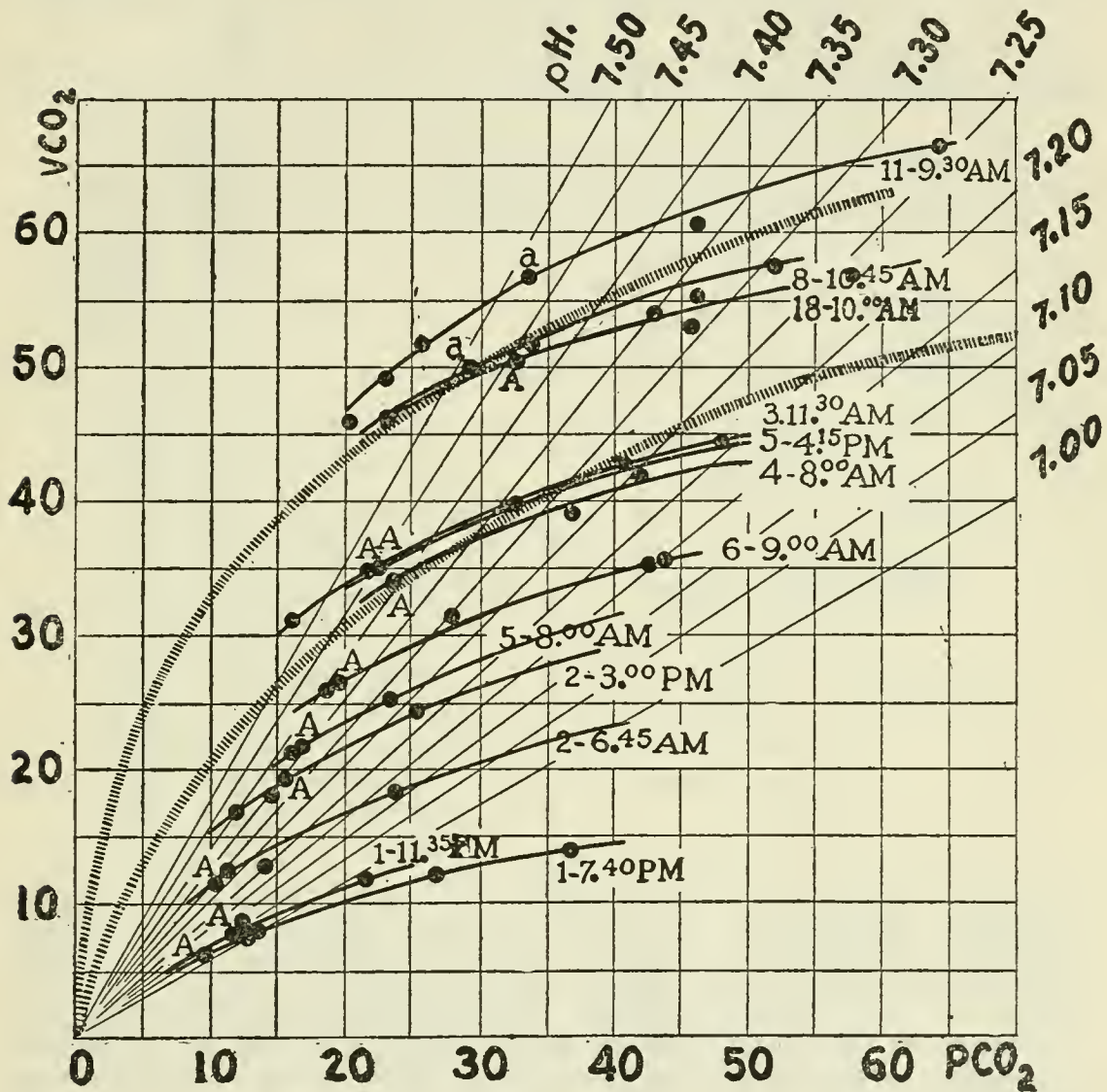


DIAGRAM NO. 4

CO<sub>2</sub> Diagram No. 4.

The following determinations of the blood fat in this case were made by Dr. Stoddard:

Jan. 29	7:30 p. m.	10.5	gm. per 100 cc. of blood.
Jan. 29	11:30 p. m.	6.27	gm. per 100 cc. of blood.
Jan. 30	6:30 a. m.	6.86	gm. per 100 cc. of blood.
Jan. 31	11:30 a. m.	4.37	gm. per 100 cc. of blood.

TABLE IX

Date 1923	Arterial CO <sub>2</sub> Vol. %	Venous CO <sub>2</sub> Vol. %	Alkali Reserve Vol. %	Alveolar CO <sub>2</sub>		pH	CO <sub>2</sub> Dissocia- tion Curve		Oxyge Capac- ity Vol. %
				A mm.	a mm.		Ten- sion mm.	Vol. %	
Jan. 29 7:40 p. m.	6.2	9.9	13.8	9.7	.....	7.03	12.8 27.0 36.8	7.5 12.2 14.1	22.5
11:35 p. m.	7.7	10.3	.....	11.8	.....	7.04	12.4 12.3 21.6	7.8 8.65 12.0	.....
30 6:45 a. m.	11.75	17.1	22.6	10.5	.....	7.29	10.4 11.3 14.3	11.5 12.5 12.8	.....
3:00 p. m.	19.3	.....	.....	15.7	.....	7.34	23.9 11.9 14.7	18.3 16.8 18.1	.....
31 11:30 a. m.	34.6	36.2	42.85	21.4	.....	7.46	25.6 16.4 21.6	24.4 31.1 34.8	.....
Feb. 1 8:00 a. m.	33.96	.....	.....	23.5	23.8	7.41	40.6 23.75 36.8	42.8 34.0 39.0	.....
2 8:00 a. m.	21.7	.....	.....	16.9	17.2	7.36	42.0 48.2 16.1	41.8 44.5 21.1	.....
4:15 p. m.	35	.....	.....	.....	22.3	7.44	23.3 32.7	25.2 39.6	.....
8:00 p. m.	.....	.....	.....	.....	24.7	.....	.....	.....	.....
3 9:00 a. m.	26.5	.....	.....	19.7	20.1	7.38	18.74 28.06 42.7	25.9 31.5 35.2	.....
5 10:45 a. m.	50.5	52.8	.....	.....	29.3	7.49	43.7 23.0 33.8	35.6 49.3 51.8	.....
15	50.5	55.1	65	32.5	32.5	7.44	43.1 52.0 20.32	54.0 57.8 46.0	13.8
							22.9 45.4 46.1	46.4 52.7 55.7	
							43.8 57.8	53.6 56.7	

Case 5. Hospital No. W. M. 253111. This patient, a girl of fifteen years, had a negative past history and a family history not remarkable except for a tendency to obesity. Three months before admission, she developed an increasing appetite, and a month later loss of weight and weakness aroused the fear of tuberculosis. Her mother gave her a high carbohydrate and high calory diet. Loss of weight and increasing weakness continued; polyuria and polydipsia became pronounced, 6.5 quarts of urine having been measured in 24 hours, five days before admission. On the morning of entrance to hospital, she developed rapidly increasing stupor.

When first seen, she had an extreme hyperpnea, a respiratory rate of 40, with increase in the respiratory excursion, a pulse of 120, temperature 98°, and a strong odor of acetone on the breath. When undisturbed, she remained in stupor, but could be aroused by stimulation to take fluids, and when aroused she was irrational. The face was flushed, the lips bright red, and the eyeballs soft.



The patient was referred to the hospital by Dr. B. H. Ragle, who supplied the insulin used and who supervised the initial treatment. In the first nine hours, she was given a total of 34 units of insulin and 63 grams of carbohydrate. Bicarbonate of soda was given to the extent of 76 grams during the first two days. Subsequent dietary measures, urinary findings and blood sugar determinations are recorded in Table X. Very little

TABLE X

Date 1923	In- sulin Units	Diet			Sodium Bicar- bonate gm.	Diacetic Acid	Urine		Blood Sugar mg. per 100 cc.
		C	P	F			Nitrogen gm.	Sugar gm.	
Nov. 16 5:00 p. m. to 12 m.	34	63	7	3	32	++++	-----	60	5:00 p. m. 338 8:00 p. m. 374 12:00 p. m. 392
Jan. 17	68	123	6	3	44	+	-----	52	8:00 a. m. 247 4:20 p. m. 212
18	44	60	30	0	-----	Trace	-----	23.5	244
19	18	40	30	0	-----	0	-----	43.8	120
20	6	45	25	0	-----	+	16.43	102	8:30 a. m. 192 4:30 p. m. 185
21	0	50	35	0	-----	+	-----	37	198
22	0	20	35	0	-----	+	-----	26	175
23	0	20	35	0	-----	+	8.0	19	153
24	0	20	35	0	-----	+	9.6	14.9	151
Feb. 1	---	30	35	35	-----	+	5.5	0	90
2	---	40	35	40	-----	+	5.65	0	-----
3	---	40	35	50	-----	0	-----	-----	-----
4	---	45	45	50	-----	+	2.95	0	-----
5	---	50	45	50	-----	+	-----	0	83
13	---	85	60	60	-----	0	-----	-----	91
25	---	110	75	110	-----	-----	-----	-----	-----

change could be observed in the patient until about 4:00 a. m. of the day after admission, when a sudden change in character of respirations occurred, the respirations dropping rapidly to a normal rate and character. By 8:00 a. m., she was sitting up in bed, was quite bright and said that she felt well except for a sense of weakness.

Since the supply of insulin was limited, its use had to be given up on the fifth day, coincident with which there was a return of moderate acidosis. The data of the fifth day are very instructive. On this day, having received only 6 units of insulin, on a diet of C. 45, P. 25, F. O., with a urine nitrogen of 16.43 grams, it is estimated that she had available 105 grams of glucose; of this she excreted in her urine 102 grams. Her D:N ratio was approximately 3.47 to 1. The small amount of sugar that she burned can be accounted for by the insulin and without it she probably would have been a complete diabetic. After this day, she developed a positive sugar balance. She became sugar free on the twelfth, had a normal blood sugar on the thirteenth day and attained nitrogen equilibrium on the sixteenth day. From this point, her diet was increased until on C. 175, P. 75, F. 100 she showed a slight trace of sugar. Her weight was

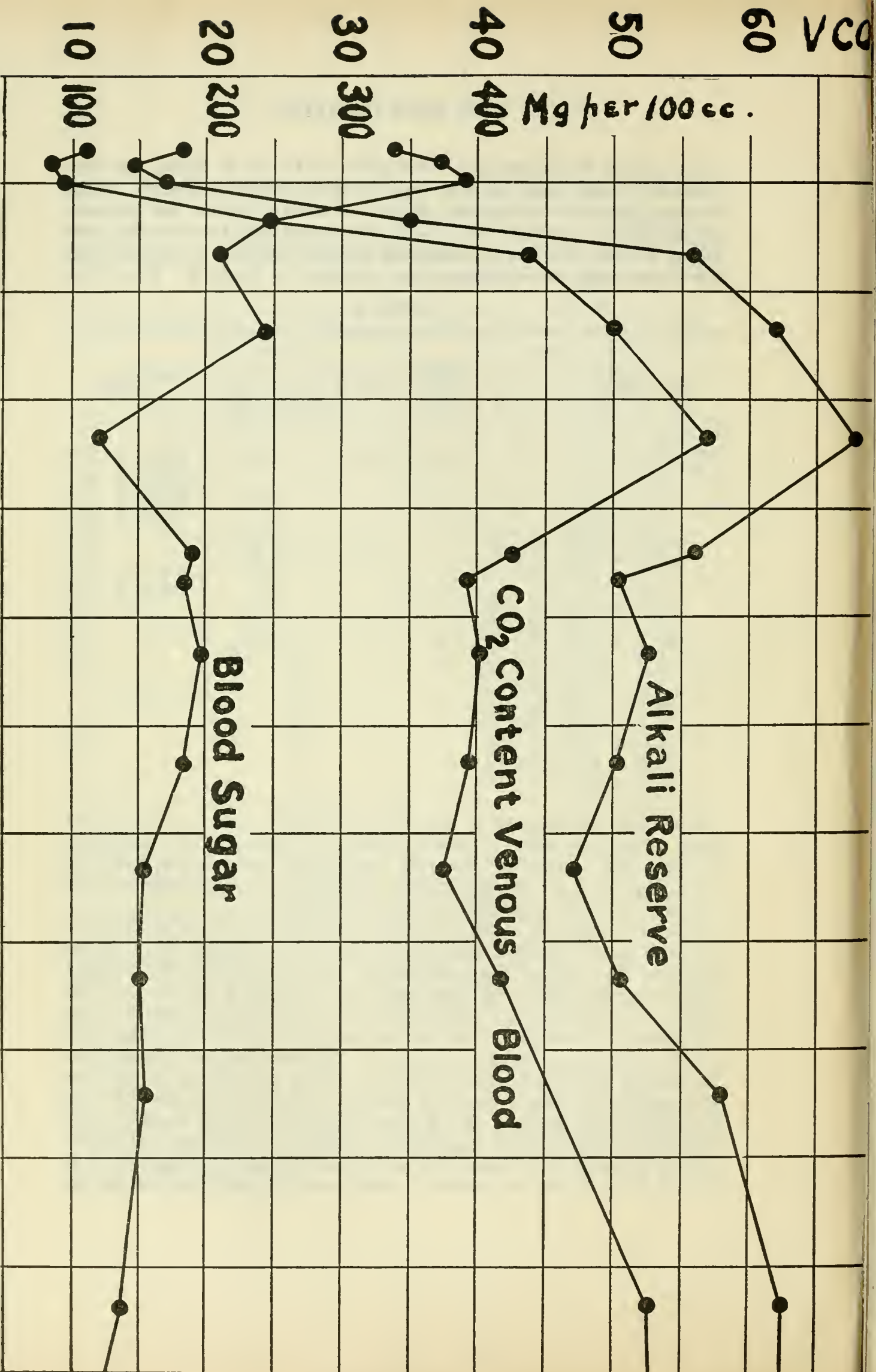


CHART NO. 2

42 kilograms on admission; on January 15, it was 40 kilograms; and on January 29, 41.5 kilograms. Data with reference to insulin, diet, etc., are shown in Table X.

#### CHART NO. 2

Chart No. 2. The points on the curve marked "Alkali Reserve" were determined in the usual manner after saturation of the plasma with  $\text{CO}_2$  at normal alveolar  $\text{CO}_2$  tension. The curve marked " $\text{CO}_2$  content Venous Blood" is drawn through points representing  $\text{CO}_2$  content of venous blood as drawn from the arm vein without stasis. The values for both curves are expressed in volumes per cent. The "Blood Sugar" curve is expressed in milligrams per 100 cc. of blood.

*Laboratory Data.* This patient, although the first in this group to be treated with insulin, is discussed last because of the slightly different nature of the laboratory data. The  $\text{CO}_2$  contents of the specimens of venous blood and the corresponding alkali reserves were determined and these are shown in Chart No. 2, together with the blood sugar determinations.

It will be evident on inspection of the chart that after the first few hours the rapid change in the  $\text{CO}_2$  of the blood is comparable to a similar change in the first three cases. With a venous  $\text{CO}_2$  as low as 8.5 volumes per cent., the arterial blood must have contained approximately 7 volumes per cent. For a matter of three hours after treatment was begun there was a decrease in venous  $\text{CO}_2$  content and only a very slight increase again after a total of seven hours. Two possibilities arise to account for this: either insufficient insulin was given to take care of rapidly forming ketones, or there may have been present an unknown amount of the same acid as was found in Case 4, which was taken care of by the continued administration of alkali. Whether without the use of alkali the same remarkable increase in blood  $\text{CO}_2$  would have occurred may be questioned, but we can say that alkali in the amount used in this case certainly did no harm and may have contributed materially to the good result obtained. The moderate degree of acidosis appearing on the fourth day, as indicated by the lower level of the curves shown in the chart, probably would not have occurred had we had sufficient insulin to have continued its use for several days longer. The data for venous  $\text{CO}_2$  content, alkali reserve and oxygen capacity are shown in Table XI.



TABLE XI

Date		Venous CO <sub>2</sub> Content Vol. %	Alkali Reserve Vol. %	Oxygen Capacity Vol. %
Jan.	16 5:00 p. m.....	11.1	18.1	21.8
	8:00 p. m.....	8.5	14.6	.....
	12:00 p. m.....	9.4	16.9	.....
17	8:00 a. m.....	24.3	35.0	.....
	4:20 p. m.....	43.8	55.8	.....
18	.....	50.1	61.9	.....
19	.....	56.8	68.0	.....
20	8:30 a. m.....	42.5	56.0	18.1
	4:30 p. m.....	39.3	50.3	.....
21	.....	40.3	52.6	.....
22	.....	39.5	50.2	15.8
23	.....	37.5	47.1	.....
24	.....	41.8	50.5	.....
25	.....	.....	58.0	.....
27	.....	52.6	67.5	.....
Feb.	1.....	53.5	67.1	.....
	13.....	55.1	67.4	.....

## DISCUSSION

*The Administration of Insulin.* The amount and rate of administration of insulin in these cases have varied greatly. In Case 5, our first case, we proceeded with more caution than in the subsequent cases, giving the patient 8 units every two hours during the first twenty-four. Case 1 was given 10 units intramuscularly and 20 units intravenously at once, followed by 10 units an hour for two doses, then 10 units every two hours. Case 2 was given 10 units an hour for five hours, then every two hours. Case 3 received an initial dose of 15 units, then 10 units every hour for six hours, then every two hours. Case 4 received 15 units each hour for four hours, then 10 units every hour. The total amount of insulin given in each case is recorded in the tables.

During the period of eight or ten hours after treatment with insulin has been started and when most of the ketones are being eliminated or destroyed, we have administered from 50 to 100 units of insulin. For two or three days following this period it seems essential in our experience to give from 30 to 50 units daily, since the patient recovering from coma has very little if any sugar metabolism for a period approximating this time. Subsequently sufficient insulin should be given to enable the patient to take a diet capable of maintaining nitrogen equilibrium. After a period of ten days or two weeks on such a regime an

attempt should be made to determine the carbohydrate tolerance without the use of insulin. We overstepped the amount of insulin necessary in Case 4 by about 150 units, failing to realize at the time that the action of insulin might be negligible when acids other than ketones are present.

The clinical experience and experimental work of Banting and his associates<sup>18</sup> appear to demonstrate that insulin promotes the combustion of glucose. It therefore follows that carbohydrate should always be available in the body when insulin is being employed. During the comatose state we gave about 1 gram of carbohydrate for each unit of insulin injected. After the coma has cleared up, as much as four grams of carbohydrate may be burned with one unit of insulin, or else other features of an improved metabolism account for the increase. A safe guide to follow would be to have a small amount of sugar appearing in the urine during the time when insulin is being pushed.

*The Use of Alkali in Diabetic Coma.* The objection to the administration of alkali is directed chiefly against the promiscuous use of it, while the argument for its use is based on the fact that normally bicarbonate of soda is one of the most important elements in the blood and necessity demands a replacement of it when there has been a serious loss.

Our experience in the treatment of five successive cases of coma leads us to advocate the restricted use of bicarbonate of soda in diabetic acidosis on grounds that we believe are rational, safe, and essential for the patient's welfare. Four of five cases received alkali, three in very small amounts and one a total of 76 grams. Each of these four might have done as well without alkali as with it so far as our evidence goes. In the remaining case, on the other hand, the failure to respond to insulin therapy and the presence of large amounts of unidentified organic acid were strong indications for the use of soda. Until it is possible to differentiate clinically the latter type of case from the others, it seems advisable to use alkali in the early hours and days of treatment in every case of coma. If frequent examinations of the blood for  $\text{CO}_2$  are possible, alkali need not be given unless it is found after four or five hours of insulin therapy that the  $\text{CO}_2$  of the blood has not increased. This delay, however, may be attended with some risk. If the patient is not in hospital alkali should be given until the danger from acidosis is passed.



The manner of administration and dosage of alkali are important considerations. We have employed in general a regime advocated by Stillman<sup>19</sup> in which 3 grams of bicarbonate of soda dissolved in a tumbler of water are given by mouth every hour. If it may not be given by mouth we gave 5 grams dissolved in 6 ounces of water by rectal injection every two or three hours. Alkali may be given until the acidosis has essentially cleared up. It is not necessary to continue giving alkali until the reaction of the urine is alkaline, but it must not be continued if the urine is no longer acid in reaction.<sup>20</sup> In no case of this series did vomiting result from the use of alkali. We feel that the intravenous use of bicarbonate of soda, except in small amounts, is contraindicated on grounds mentioned by Allen, Stillman and Fitz:<sup>21</sup> "It is probably bad policy to try to force a low blood alkalinity suddenly up to or above normal by large alkali dosage, especially intravenously. Progress is favorable if the level of the plasma bicarbonate tends distinctly though gradually upward." With the use of insulin large doses of alkali appear to be unnecessary. The object to be accomplished can be met with an amount of soda approximating 25 to 40 grams a day, and this need not be continued for more than two or three days in most cases.

*The Determination of Alveolar CO<sub>2</sub>.* The tension of alveolar CO<sub>2</sub> as determined from the A points on the CO<sub>2</sub> dissociation curves in this series ranged from 8 to 12 mm. of mercury. The only objection that may be raised to these figures is that the tension of CO<sub>2</sub> in the arterial blood is not in equilibrium with that of the alveolar CO<sub>2</sub>, but the existence of this equilibrium, except in such cases as cardiac failure of the congestive type or in the presence of certain pulmonary lesions, is generally accepted. However, Peters, Barr and Rule<sup>6</sup> conclude from their data that in normal subjects there may be as great a difference as 11 mm. between the tension of CO<sub>2</sub> in alveolar air and arterial blood. We have made a number of determinations of alveolar CO<sub>2</sub> tension, using the Haldane tube, and taking samples of air at the end of expiration only just before arterial blood was drawn. Enough of these are given in Table XII to show that only a small difference if any exists between alveolar CO<sub>2</sub> and arterial CO<sub>2</sub>. It therefore appears reasonable to believe that the figures as given above for alveolar CO<sub>2</sub> tension in coma are correct.



TABLE XII

Case	Date	Alveolar CO <sub>2</sub> Tension	
		Determined From A Point mm.	Determined With Aid of Haldane Tube mm.
2	Jan. 12	26.4	26.2
	25	34.2	35.7
3	17	26	27.2
	18	37.8	37.0
	26	39.8	40.7
	Feb. 6	38	40.7
4	1	23.5	23.8
	2	16.9	17.2
	3	19.7	20.1

All attempts to determine alveolar CO<sub>2</sub> tension of patients in coma by methods requiring samples of alveolar air have led to inaccurate results because intelligent co-operation of the patient is a prerequisite in obtaining proper air samples. The determination of the tension of CO<sub>2</sub> in arterial blood is quite accurate, but the technique is more involved than is the case with alveolar air methods.

*The Reaction of the Blood.* The figures for the pH of the arterial blood in the four cases in which it was determined were 7.20, 7.17, 7.17 and 7.03 at the time of the first observation. It is possible that there is a slight error in the pH calculations in this range, but the error is such that the real pH will be even less than these estimates. The fifth case probably approached the lowest of these figures at the time when the venous CO<sub>2</sub> content was 8.5 volumes per cent. These reactions of the blood are at such low levels that a very small shift in blood alkali causes a comparatively great shift in pH. Cases 1 and 2, in whom the pH was found to be 7.17, had CO<sub>2</sub> dissociation curves which at a tension of 10 mm. of mercury were only 1 volume per cent. higher than the curve of Case 4 when the pH in this case was only 7.03. Reference to Chart No. 3, in which arterial CO<sub>2</sub>, pH, and alveolar CO<sub>2</sub> are plotted against the time at which the observations were made, may serve to clear up what appears to be a very complex mechanism. It will be noted that between the second and third observations a very small change in the CO<sub>2</sub> of the blood produced a great change in pH, with the alveolar CO<sub>2</sub> still remaining at a low level. On the fifth day, however, a rather large drop in

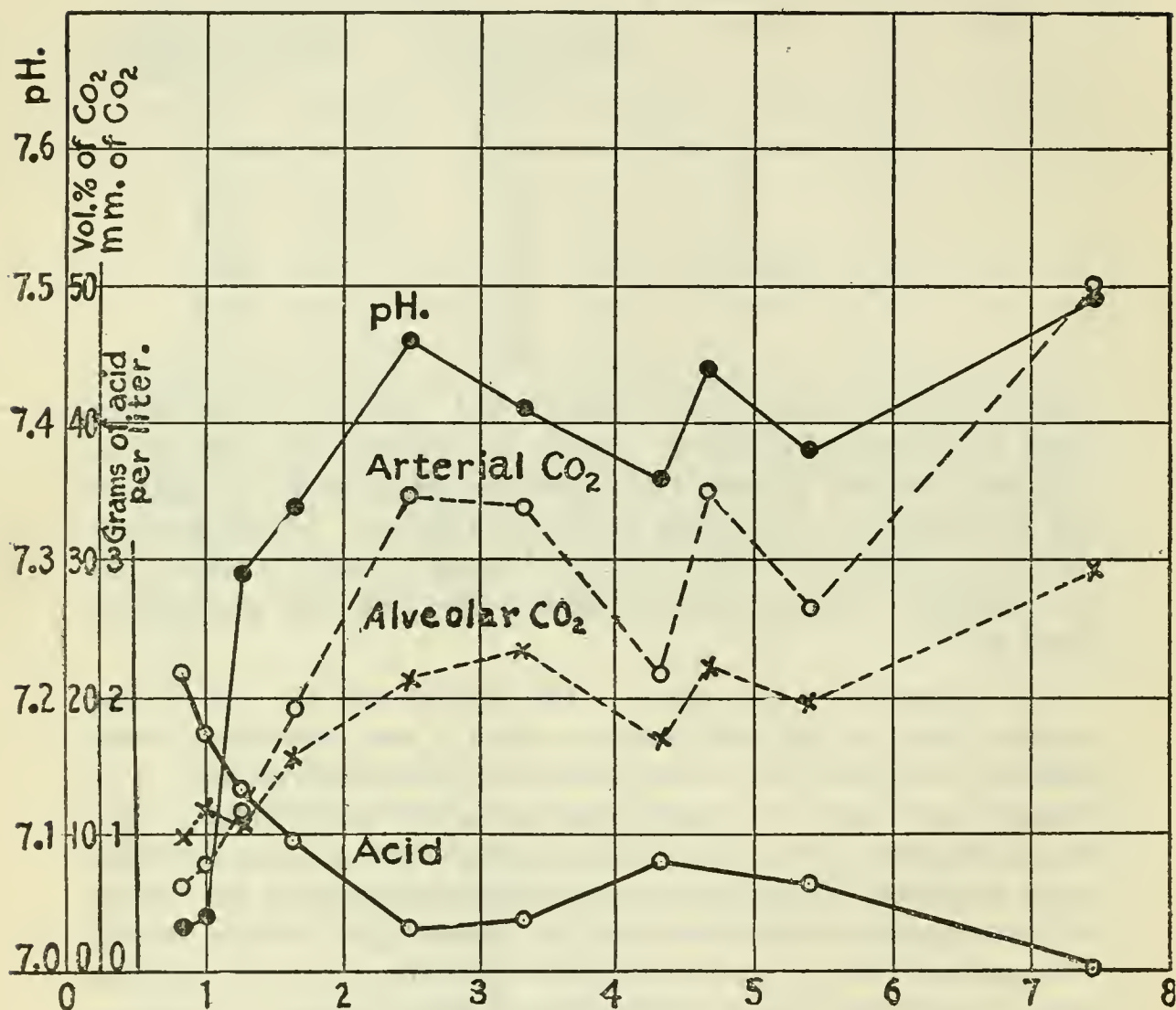


CHART NO. 3

Chart No. 3. The data for pH, arterial CO<sub>2</sub>, and alveolar CO<sub>2</sub> tension are plotted to show the existing relationships as time progressed. The early changes in the slope of the curves reflects the recovery from acidosis in a manner similar to the rise in the level of the CO<sub>2</sub> dissociation curves shown in the Diagrams. The effect on pH of small and more nearly normal amounts of alkali as represented in the arterial CO<sub>2</sub> curve is well shown. The "Acid" curve attempts to reflect the rate of disappearance of acid from the body.

arterial  $\text{CO}_2$  caused but a small shift in pH, which may be accounted for through better buffering of the blood and better respiratory control of  $\text{CO}_2$ .

The sloping curve of blood is an expression of the fact that hemoglobin and other buffers give up base to carbonic acid when the tension of the latter is increased, thus forming sodium bicarbonate. The first effect of abnormal acids is to reduce the sodium bicarbonate, an effect which can be compensated by blowing off  $\text{CO}_2$  in the lungs, and it does not make a large change in the buffering of the blood. When the abnormal acid is present in quantity, it robs the proteins of sodium and upsets the normal mechanism of buffering. This is shown by the curves, for the pH under these conditions varies greatly as the  $\text{CO}_2$  tension changes. The  $\text{CO}_2$  curves are flattened out when much organic acid is present.

*The Amount of Acid in the Body.* Aside from clinical phenomena the usually accepted method of measuring the degree of acidosis present is that of determining the amount of  $\text{CO}_2$  in whole blood as drawn or in plasma after saturation at a constant  $\text{CO}_2$  tension. The figure thus obtained, while expressed in terms of volumes per cent. of  $\text{CO}_2$ , is also taken to express the amount of available alkali since the  $\text{CO}_2$  is carried as bicarbonate. In reality a number of changes have taken place. The accumulation of organic acids must displace anions other than bicarbonate and remove base from the proteins. Such details cannot be studied without exact determinations of almost all the substances in blood, but a general idea of the effect of acidosis can be obtained by a very simple method.

A specimen of normal blood was taken and known amounts of acetic acid were added to different portions, and the  $\text{CO}_2$  bound at 40 mm. of mercury was determined. The experiment parallels that of Van Slyke, Stillman and Cullen,<sup>22</sup> in which acetic acid was added to plasma and the amount of  $\text{CO}_2$  bound as bicarbonate determined. The data of our experiment are given in Table XIII and are plotted in Figure 1. The curve thus obtained was used to find the amount of acid corresponding to the  $\text{CO}_2$  bound at 40 mm. in each of our patients. If the patient had a normal blood bicarbonate above 46 volumes per cent., the figure in our normal control with no acid, the acid required to bring the blood to 46 volumes per cent. was calculated and added to 46.



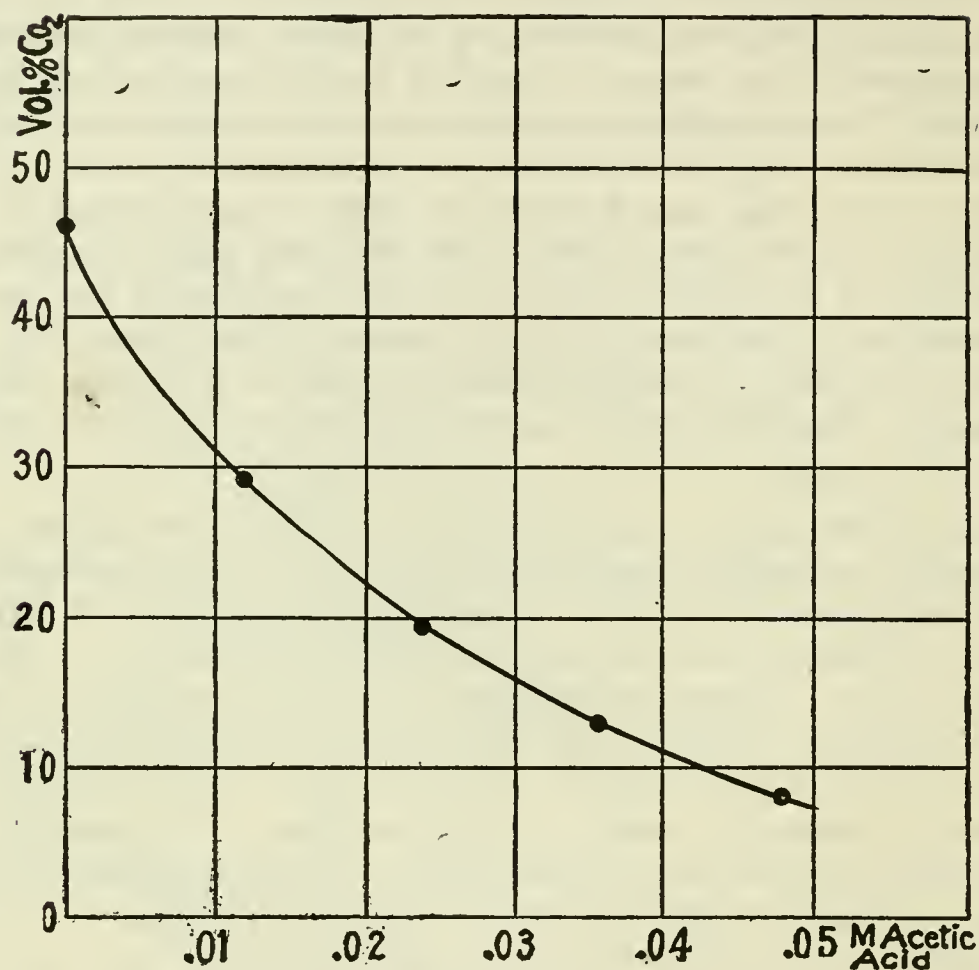


FIGURE NO. 1

Fig. 1. This curve represents the fall in CO<sub>2</sub> combining power of whole blood at a tension of 40 mm., with additions of increasing amounts of acid. The steepness of the curve between 0 and .02 M acid is due to the combination with acid of practically all of the free alkali bicarbonate. The lower portion of the curve flattens out because of the influence of protein bicarbonate and other protein buffers. It is a titration curve by means of which the molecular concentration of acid in the body may be determined if the CO<sub>2</sub> content of the blood at a tension of 40 mm. of mercury is known.

The changes which take place in the blood as a tissue of the body are far too subtle for representation in the simple manner we have described. The risks of error in the acid calculation are fully admitted, but it seems justifiable to take them in order to give a provisional quantitative explanation of the phenomena. It may be noted that a very similar indirect method of measuring acid has been found useful in previous work, the oxygen affinity method described by Barcroft,<sup>23</sup> by means of which it was found that exercise at high altitudes gave about 12 per cent. of the acid present in diabetic coma. In our work it was simpler to use the

TABLE XIII  
Normal Blood of Dr. L. W.  
Oxygen Capacity 18.4 volumes per cent.

No.	Concentration of Acetic Acid Added to Blood	CO <sub>2</sub> Tension mm. of Mercury	Vol. % of CO <sub>2</sub> Observed	Vol % of CO <sub>2</sub> at 40 mm. Interpolated
1	0	45.7	48.4	46.0
2	.012 M	42.7	30.0	29.3
3	.0239 M	40.4	19.6	19.5
4	.0359 M	46.3	13.5	13.0
5	.0479 M	44.5	8.9	8.2

CO<sub>2</sub> method because the measurements from which the calculations were made were required in any case for the construction of the CO<sub>2</sub> diagrams.

Apart from discussions of the accuracy of any particular method, it seems a matter of real importance to give quantitative estimations of acidosis in terms of acid as well as in terms of bicarbonate. There are two advantages in the former statement. First, the bicarbonate concentration is a variable, changing with the CO<sub>2</sub> tension, while the amount of abnormal acid present is a constant in any blood sample. The second reason is that in theory the cause of acidosis is the accumulation of unburnt organic acids, and therefore a measurement of the concentration of these bodies is the simplest quantitative method of characterizing acidosis. The calculation of the acid value with the aid of the curve shown in Figure 1, given the CO<sub>2</sub> bound at 40 mm. of mercury, takes no longer than the calculation of the CO<sub>2</sub> volume itself. The estimations of the molecular concentrations of acid are based on the level of the CO<sub>2</sub> curves at 40 mm. and are given in Table XIV.

It is interesting to compare the results shown in Table XIV with estimates of the total available base present in the blood. Van Slyke<sup>24</sup> gives 0.032 M.; Parsons<sup>25</sup> gives 0.045 M. In one of our patients the acid value was about the same as the total available base in the former estimate.

TABLE XIV

Case	Day	Time	Vol. % of CO <sub>2</sub> at 40 mm. From Disso- ciation Curve	Estimated Equivalent of Acid, Read From Curve, Fig. 1.
1	1	7:30 p. m.	17.0	.031 M
	2	7:30 a. m.	32.5	.012 M
	3	12:00 noon	45.5	.003 M
	5	11:30 a. m.	50.0	0.0
2	1	11:30 a. m.	15.5	.031 M
	2	8:00 a. m.	41.5	.003 M
	16	9:30 a. m.	48.0	0.0
3	1	9:00 a. m.	20.5	.027 M
	2	11:00 a. m.	46.0	.005 M
	3	10:00 a. m.	43.0	.006 M
	4	9:30 a. m.	52.0	.001 M
	11	9:45 a. m.	54.0	0.0
4	1	7:40 p. m.	14.5	.037 M
	1	11:30 p. m.	16.5	.033 M
	2	6:45 a. m.	23.0	.022 M
	2	3:00 p. m.	29.0	.016 M
	3	11:30 a. m.	42.5	.006 M
	4	8:00 a. m.	41.0	.006 M
	5	8:00 a. m.	31.5	.014 M
	6	9:00 a. m.	34.5	.011 M
	8	10:45 a. m.	54.5	0.0

We may now attempt a rough estimate of the total amount of acid present in the body in each of the four cases of coma in which the data permit of such a calculation. The data used in these estimations are given below in full, so that corrections can be made when more is known of the distribution of acids in the body. We have assumed that the acid or acids in question are evenly distributed throughout the body fluids.<sup>26</sup> The body fluids have been taken as 70 per cent. of the body weight, and the concentration of acid has been multiplied by this volume. On account of the state of dehydration present in these patients, the estimate of body fluid may be rather too large.

It is convenient to have figures in grams as well as gram molecules; therefore the figures in column 4 have been multiplied by 103, the molecular weight of diacetic acid, which may be taken as a type of the nonvolatile acids present.



It is also of interest to compare at constant pH the fall in bicarbonate with its equivalent of acid as calculated from the curve of Figure 1. In Case 4, on admission the  $\text{CO}_2$  volume at pH 7.3 was 5.5; the normal was 46.5 volumes per cent. The fall in  $\text{CO}_2$  was 41 volumes per cent., corresponding to a molecular concentra-

TABLE XV

Case	Weight in Kg.	Body Fluid in Liters	Concen- tration of Acid	Total Acid in Gram Mols.	Total Acid in Grams
No. 1	32	22.5	.031	.698	71.0
2	42	28.8	.031	.892	91.0
3	40	28.0	.027	.757	77.2
4	39	27.3	.037	1.01	103.0

tion of 0.018 M, while the actual change in acid value as read off from the curve of Figure 1 was 0.036 M. This discrepancy shows that the diminution in bicarbonate is not a valid measurement of the amount of acid in the blood. The bicarbonate calculation would be correct if no  $\text{CO}_2$  combined with the proteins and if the buffer action of hemoglobin was independent of the bicarbonate concentration. The data of Van Slyke and Cullen<sup>26</sup> and experimental data (to be published shortly) show that both of these assumptions are unsound. The acid value 0.036 M is probably a little too high, but it gives a better idea of the state of affairs than the 0.018 M estimate.

The writers wish to express their appreciation of the interest taken in this work by Professor L. J. Henderson, Dr. Roger I. Lee and Dr. J. H. Means, whose many suggestions have been helpful. They are also indebted to Dr. E. P. Joslin, Dr. B. H. Ragle, and Dr. F. Gorham Brigham for the supply of insulin used, and especially to Dr. Ragle for referring to the hospital the first case of this series and for his direction of the treatment instituted in this patient. The other cases were referred by Dr. Brigham, Dr. H. W. Goodall, and Dr. H. H. Amiral. The close co-operation of the house staff has made the work possible.

#### ADDENDUM

Since the above was written two patients with diabetes, one in coma and one in a pre-comatose state, entered the hospital and died; the first, two hours after admission; the second, after thirty-five hours.

The first patient, a woman of 50, had been drowsy for three or four days and in deep coma with marked hyperpnea for at least several hours before admission. The  $\text{CO}_2$  content of her arterial blood was 7.2 volumes per cent., and pH 7.07, and the alveolar  $\text{CO}_2$  tension 10.6 mm. of mercury. In spite of 45 units of insulin, she died two hours after admission. Autopsy examination was essentially negative.

The second patient was a man of 24, with a history of diabetes for two years, and was known to have had glycosuria almost continuously for one year. On admission, he presented evidence of great prostration, hyperpnea of moderate degree, and heavy acetone odor on his breath, but he was still able to answer questions intelligently. There was 3.3% of sugar in the urine and a +++ ferric chloride reaction. He was given 20 units of insulin subcutaneously at once, followed by 15 units every hour for five doses, then 10 units every three hours. The arterial  $\text{CO}_2$  was 6.7 volumes per cent., the pH 7.06, and the alveolar  $\text{CO}_2$  tension 9 mm. of mercury. The  $\text{CO}_2$  content of the venous blood was 9.9 volumes per cent. Five hours later, after 80 units of insulin had been given, the venous  $\text{CO}_2$  had risen only to 15 volumes per cent. The odor of acetone had disappeared from the breath, and there was only a faint reaction with ferric chloride in the urine. Alkali therapy was initiated and a total of 31 grams was given up to the time of death. Twenty and one-half hours after the first observation, the arterial  $\text{CO}_2$  content was 25.5 volumes per cent., the pH 7.27, and the alveolar  $\text{CO}_2$  tension 23.8 mm. of mercury. The urine contained 3% of sugar and had no ferric chloride reaction. During the succeeding fifteen hours, the pulse steadily mounted to 180, the temperature by rectum rose to  $103^\circ$ , and the blood pressure dropped to 60/50. For a period of approximately eighteen hours before death, he complained of excessive soreness of the body, he was extremely weak, but remained rational almost up to the moment of exitus. Examination of blood obtained post mortem by auricular puncture showed an alkali reserve of 41 volumes per cent. The non-protein nitrogen of this specimen of blood was 74 mg. per 100 cc., as compared with 66.6 mg. fifteen hours previously. The urine output was 855 cc. during the first six hours on a fluid intake of 4000 cc., 270 cc. during the succeeding twenty-four hours with a fluid intake of 6000 cc., and catheterization after death yielded 110 cc.

Clinically and by laboratory tests, this patient appears to have had the same type of acidosis as was found in Case 4 reported above. Further details of analyses for acetone bodies in blood and urine, titration of urinary acidity, etc., will be published later. Permission for post mortem examination was not granted.

It seems probable that this patient died from the general effect of a prolonged period of severe acidosis. So it appears possible that had alkali therapy been instituted without delay and the period of acidosis shortened thereby, his chance of recovery might have been materially improved. The risks attendant upon withholding alkalies in such cases as are reported in this paper are too great to warrant delay in their use.

It is noteworthy that the clinical picture presented by the last patient, in contrast to all of the others, failed to indicate the extreme gravity of his condition.

### SUMMARY

A study of the acid-base equilibrium has been made in five cases of diabetic coma treated successfully with insulin. Minimal figures with recovery thus far observed are reported for the  $\text{CO}_2$  content of arterial blood, alveolar  $\text{CO}_2$  tension and pH of the blood.

A case of diabetic coma is reported in which the acidosis was in part due to organic acid or acids not of the ketone group. Thirteen cases, possibly of this same nature, have been collected from the case records of the Massachusetts General Hospital during the decade following January 1, 1912.

The use of insulin in the treatment of diabetic coma is discussed.

The administration in a restricted way of bicarbonate of soda is advised as a part of the treatment of diabetic coma.

Reference is made to the equilibrium existing between the tension of  $\text{CO}_2$  in the alveolar air and that of the arterial blood.

A discussion of the mechanism by which the reaction of the blood is controlled, from the point of view of blood buffers, is given.

An attempt has been made to estimate the molecular concentration and total amount of acid present in the cases reported.

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STUDIES OF THE THYROID APPARATUS. XXI. THE  
WATER CONTENT AND REFRACTIVE INDEX OF  
THE BLOOD-SERUM OF ALBINO RATS THYRO-  
PARATHYROIDECTOMIZED AND PARATHY-  
ROIDECTOMIZED AT 75 DAYS OF AGE

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This paper continues the report of the investigation of the relation of age and sex to the role of the thyroid apparatus in the maintenance of blood serum composition as determined by the water per cent. and the refractive index.

The details of the general plan, scope and methods of the study have been given in earlier communications. It should be noted, however, that the animals from which the values reported in this paper were obtained were from the same parent stock and had the same environment, dietary, sex and litter control as did those on which the first report was made and differed only in regard to the age at which the structures were removed (1). They are the same animals as those from which the data on the gross growth were obtained (2). The technic was the same as that previously described. From 0.7 to 1.0 cc. of serum was used for each analysis.

Table 1 contains the statistical data for the water per cent. and refractive index of the sera of the various groups. To give the individual observations and to plot them in a demonstration of differences in the refractive index: water per cent. relation would require an extension of tabulation and an increase in the number of charts which is not warranted by the limited extent to which the data are capable of being analyzed.

From this table it is evident that water content of the blood serum of the thypars of both sexes at 150 days of age did not differ to a statistically valid degree from that of their respective controls. Nevertheless, the direction of deviation of the water per cent. of the serum of the thypars from that of the controls is the same as that exhibited in the serum of the thypar males of the 100 day old series, and is opposite to that of the refractive

TABLE 1.

The statistical data for the water per cent. and refractive index of the blood serum of the eight groups of rats of the 75 day old series as observed at 150 days of age.

Animals		Water Per Cent.			Refractive Index		
Group	No.	Mean	S. D.	P.E.M.	Mean	S. D.	P. E. M.
MALES							
Ctls. for Thyphs.....	9	91.89	0.23	0.05	1.34822	0.00034	0.00008
Thypars.....	11	91.72	0.42	0.09	1.34847	0.00080	0.00016
Ctls. for Paras.....	8	91.82	0.38	0.09	1.34823	0.00053	0.00013
Parathys.....	12	92.10	0.53	0.10	1.34782	0.00108	0.00021
FEMALES							
Ctls. for Thyphs.....	10	91.72	0.51	0.11	1.34850	0.00085	0.00018
Thypars.....	12	91.67	0.66	0.13	1.34857	0.00099	0.00019
Ctls. for Paras.....	8	92.07	0.25	0.06	1.34799	0.00052	0.00012
Parathys.....	10	91.87	0.24	0.05	1.34822	0.00026	0.00006

S. D.—Standard Deviation.  
P. E. M.—Probable Error of Mean.

index. This reciprocal relationship indicates the reality of the differences, notwithstanding their statistical invalidity when taken by themselves. The lesser definiteness of the response is correlatable with the fact that the retardation of body growth was less marked in the 75 day old series than in the 100 day old series. Further support for the belief that thyroid deficiency is productive of a tendency to partial dehydration of the blood-serum is given by the fact that in the 75 day old female thypars there is no evidence of an increase in water per cent. of the blood serum, such as was exhibited in the 100 day old series, and that the reciprocal direction of deviation of water and refractive index was maintained. It will be remembered that this drift toward a higher water content of the serum in the female thypars of the 100 day old series was attributed to a condition of partial physiological inanition. The female thypars of the 75 day old series did not exhibit this absolute inhibition of growth, but increased in weight. This quantitative difference in growth response and the accompanying shift in the direction of deviation of the water per cent. and refractive index supports not only the interpretation of the sex-difference given in the earlier paper, but the general



conclusion that thyroid deficiency tends to produce a blood-serum of lower water per cent. than normal.

Turning now to the parathys, it is seen that there was a sex-difference in response. In the males, the water per cent. was increased; in the females, it was decreased. Since these changes were accompanied by the opposite change in refractive index, they are real.

This sex-difference is no more explicable, at present, than is the sex-difference in the gross growth response of these parathy rats.\* Nevertheless, the validity of its existence is established by

TABLE 2.

Comparison of the observed values for water per cent. and refractive index with the expected on the basis of physiological age determined from body length measurements.

Group	Body Length, mm.	Age, Days	Water Per Cent			Refractive Index		
	Observed	Physio-logical	Calculated	Observed	Difference	Calculated	Observed	Difference
MALES								
Ctl. Thyps	206.4	147	90.69	91.89	1.20	1.35010	1.34822	—0.00188
Thypars	186.7	92	91.40	91.73	0.33	1.34909	1.34847	—0.00062
Ctl. Paras	208.4	157	90.46	91.82	1.36	1.35012	1.34823	—0.00189
Parathys	182.2	85	91.55	92.10	0.55	1.34893	1.34782	—0.00111
FEMALES								
Ctl. Thyps	189.4	131	90.89	91.72	0.83	1.34988	1.34850	—0.00138
Thypars	170.9	83	91.52	91.67	0.15	1.34888	1.34857	—0.00031
Ctl. Paras	185.5	117	91.29	92.07	0.78	1.34960	1.34799	—0.00161
Parathys	172.3	85	91.20	91.87	0.57	1.34893	1.34822	—0.00071

the correlation. It is thus clear that the initiation of a parathyroid deficiency at 75 days of age has an effect upon the water content of blood serum, which is not made evident when the defect is produced at 100 days. It is probable that the cumulative effect of the longer exposure to the toxemia which exerts an influence upon body weight growth, is an important factor, but sex and age differences in sensitivity should not be eliminated.

A comparison of the observed values for water per cent. and refractive index, with those expected on the basis of the physiological age as determined from the observed body length, is pos-

\* This has been described and discussed in detail in an earlier paper (2).

sible from the data in Table 2. This method of analysis gives presumptive evidence that during the 75 day interval elapsing between the loss of the thyroid apparatus or the parathyroids and the time of taking the serum, the blood serum tended to become concentrated to a greater degree than it would have if the concentration due to an increasing age had followed the course as determined by growth in body length. The response was, therefore, similar to that which obtained in the 100 day old series. There was no evidence of a consistent sex-difference in water content or refractive index of the blood serum of the control rats at 150 days of age. This is a confirmation of the earlier observations. The values here reported are also in statistical agreement with those obtained from the male and female controls of the 100 day old series at 150 days of age.

In order to determine whether or not the glandular deficiencies caused a change in the nature or distribution of the refractive substances of the serum, the same method of analysis of the water per cent.-refractive index relation as used in the former paper was used here. The equations representing this relation were calculated from the individual observations by the method of least squares. They are given in Table 3.

In order to facilitate computation, the number 1.34295 was subtracted from the observed refractive indices and the remainder multiplied by 1000. From the water per cent., 90.00 was subtracted. The resulting numbers were used in the development of the equations. The substitution of arbitrary values for  $y$  (water per cent.), such as 94.00 and 91.00, in the type equation

$$x = \frac{1 - b(y - 90.00)}{\frac{a}{1000}} + 1.34295$$

gives the values for the respective refractive indices, from which the equations representing the water per cent.-refractive index relations are easily obtained.

The numerical value of the slope of the lines represented by the equations affords a convenient basis for comparison of the effect of the experimental procedures of serum constitution. This method of analysis was discussed in the first report.

TABLE 3.

The equations of the linear relationship between the water per cent. and the refractive index, the slopes of the equations, the average deviation of the observed refractive indices from the calculated and the mean ranges of the slopes.

Group	Equation	Av. Dev.	Slope	Mean Range of Slope
MALES				
Ctls. Thyps.....	$\times = \frac{1-0.0010434y}{0.670567}$	.00010	—0.6424	—0.6160 to —0.6711
Thypars.....	$\times = \frac{1-0.00122442y}{0.658289}$	.00017	—0.5376	—0.5076 to —0.5725
Ctls. Paras.....	$\times = \frac{1-0.00093356y}{0.678128}$	.00018	—0.7264	—0.6681 to —0.7958
Parathys.....	$\times = \frac{1-0.00132076y}{0.651692}$	.00010	—0.4934	—0.4777 to —0.5102
FEMALES				
Ctls. Thyps.....	$\times = \frac{1-0.0011121y}{0.665925}$	.00017	—0.5988	—0.5607 to —0.6424
Thypars.....	$\times = \frac{1-0.00128636y}{0.654079}$	.00015	—0.5085	—0.4800 to —0.5310
Ctls. Paras.....	$\times = \frac{1-0.00133399y}{0.650728}$	.00028	—0.4878	—0.4471 to —0.5367
Parathys.....	$\times = \frac{1-0.00072895y}{0.692044}$	.00012	—0.9494	—0.8824 to —1.0274

It is necessary, however, to determine whether the differences in the slope values are valid and hence significant. It is obvious that the individual observations deviate to greater or lesser degree from their equation. The average of the sum (regardless of sign) of the individual deviations of the observed refractive indices from those calculated by the substitution of the corresponding water per cent. in the equation for the group, is analagous to the standard deviation used in statistical computations. It represents the mean of the range of variability of the



observed water per cent.-refractive index relation and is obviously greater than the probable error. If, therefore, the maximum and minimum slopes are calculated from the group equation in which the average deviation is adequately expressed, the values obtained represent, to a satisfactory degree, the maximum limits, or range, within which the slope representing the water per cent.-refractive index relation can be expected to vary for that particular group. They thus allow a determination of the validity of the differences in slope exhibited by the several groups of observations. The various computations have been made and the results given in Table 3. Differences in slopes are interpreted as indicating differences in the nature or distribution of the refractive substances other than water.

It is seen from Table 3 that the loss of the thyroid apparatus at 75 days of age produced a definite, though small distortion of blood-serum composition in both sexes, and to about the same degree in each. This response was not shown when thyro-parathyroidectomy was done at 100 days of age. There is, therefore, an age difference in the response to the glandular deficiency.

The initiation of the toxemia of parathyroid deficiency at 75 days of age caused a marked distortion in the blood-serum composition, which was much greater than that caused by the combined thyroid-parathyroid deficiency. The reactions of the males was opposite from that of the females. The reaction of both sexes was greater in extent than that exhibited by the rats of the 100 day old series.

The sex difference in type of response, as demonstrated by this method of analysis, falls in line with the sex difference in the mean water per cent. and refractive index, and the sex difference in body weight growth. Although the differences are at present inexplicable, the occurrence of opposite reaction types in the two sexes in these three different measurements is indubitable evidence of the existence of a sex-specific mechanism of resistance to the toxemia of parathyroid deficiency, at the age of 75 days, which is not participating to a demonstrable extent at 100 days of age. This phase of the problem will be more extensively analyzed in a future communication.

The apparent greater distortion of blood-serum composition, following parathyroid loss at 75 days of age, can be attributed to

the cumulative effect of the toxemia over the longer period, since evidence of such effect in retardation of growth in body weight is clear (2). In view of this, and the fact that no distortion of blood-serum composition was shown in the male thyphars of the 100 day old series, it is possible that the slight distortion exhibited in the thyphars of both sexes in the 75 day old series was rather an expression of the parathyroid deficiency than of thyroid deprivation.

The thyphar controls show no sex difference or difference from the controls of the 100 day old series in the nature or distribution of the refractive substances at 150 days of age. The parathy controls of the 75 day old series show a slight sex difference. The female parathy controls differ from the thyphar controls in this series to a slight extent, while in the males differences are not establishable. The explanation for these differences is not at hand.

#### *Summary and Conclusions*

Since the text contains a detailed description of the observations, only the general conclusions of the results of the study need be given here.

The earlier conclusion that the thyroid gland is probably concerned in the fluid exchange of the body is confirmed. It appears, however, as if the age at which the thyroid deficiency is initiated is a factor in the extent of the tendency to anhydremia, in that the rats deprived of the thyroid apparatus at 75 days of age did not show as marked a lowering of the water content of the blood serum as did the males from which the structure was removed at 100 days of age.

Confirmation is also had of the earlier conclusion that the toxemia of parathyroid deficiency causes a disturbance in the nature or distribution of the refractive substances aside from water in the blood serum. The extent of the distortion was greater than that exhibited by the 100 day old series, probably because of the cumulative action of the toxemia over the longer period. This disturbance is apparently sex specific in type in rats deprived of the glands at 75 days of age, in that the direction of change is apparently the opposite in the males from what it is in the females. This opposite type of reaction is also shown in the mean water per cent. and refractive index.

Age and sex are thus shown to be factors of influences in the reaction of the blood-serum to thyroid and parathyroid deficiencies.

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## DR. JACOB ROSENBLOOM

On September 25, 1923, there died in Pittsburgh Dr. Jacob Rosenbloom.

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I met Dr. Rosenbloom thirteen years ago when he was twenty-five years old. At that time, he was a living dynamo working fifteen to eighteen hours daily in the laboratory on several problems in biochemical research simultaneously. His mind was one of the alertest that I have known. He constantly read the scientific literature published all over the world, and with the most tenacious memory retained and indexed his gleanings so that he could throw light at any moment on any problem in the very diverse fields of medicine and biochemistry.

His tastes were catholic; his reading was as diverse as literature itself. He spent very little time in the enjoyment of the trivialities of life. His library and his laboratory were his places of recreation and repose.

Dr. Rosenbloom was born at Braddock, Pa., on February 25, 1884. He received his elementary and high school education in the local schools and then entered the University of Western Pennsylvania, from which he graduated in 1905, with the degree of Bachelor of Science. His professor at the university was Dr. Francis Phillips, a man who has left his mark on American chemistry. Professor Phillips prophesied a brilliant future for Dr. Rosenbloom's chemical attainments and he remained Dr. Rosenbloom's friend and admirer until his own demise. From Columbia, Dr. Rosenbloom received the degrees of Doctor of Medicine and Doctor of Philosophy. Later on he was appointed Biochemist in the Western Pennsylvania Hospital of Pittsburgh.

His specialty in medicine was the Diseases of Metabolism. He was the first man in the United States to recognize such a specialty, to enter it and to find many imitators.

Dr. Rosenbloom was generous to a fault. His time, his purse and his labors were always at the command of his friends. One can conceive of the generosity of his mind when one is told that knowing that his time for research was limited, he published at his own expense a brochure entitled "*1,000 PROBLEMS IN BIOCHEMICAL RESEARCH*," and freely distributed it to his friends and his enemies for them to grasp these suggestions and to work out these original thoughts of his.

He has contributed more than one hundred reports of original research in the various medical and biochemical journals of America, England and Germany. Those who have read his works will feel greatly the loss that science sustains.

Toward the later years of his young life, Dr. Rosenbloom devoted much attention to the history of medicine and he has made several interesting contributions to that subject in the "Annals of Medicine" and in "Medical Life." He has asked the author of these lines before he died, not knowing that he was going to die, to collaborate with him in the publication of a volume on "*CRITICAL STUDIES IN THE HISTORY OF MEDICINE*." This volume will soon be presented for publication.

MAX KAHN.



# A STUDY OF THE EFFECTS OF RADIUM ON METABOLISM.

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#### I. Review of literature.

- (a) Effects of radium on metabolism of animals.

Berg and Welker studied the effect of very small doses of radium on the metabolism of dogs. They concluded that the ingestion of radium per os was without any special influence on metabolism. In one experiment there was a slight increase in the nitrogen output but in another experiment it was decreased to about the same degree. They noted an increased output of urine.

Theis and Bagg have found that the injection of an active deposit of radium into dogs is followed by an increased output of nitrogen and urea. The ammonia nitrogen was decidedly increased. The creatinine and uric acid were also increased. The



dogs were given doses of two to six millicuries per kilogram of weight.

Höchendorff found that in dogs on the day following the injection of mono-sodium-urate, the excretion of total nitrogen showed a constant increase, when the dogs were kept under the action of radium emanation. This finding persisted for eight to nine days. He also found that the allantoin of the urine was increased.

(b) Effects of radium on metabolism of man in health and disease.

Very little work has been carried out in this interesting and important field. The following abstracts are all that a careful search of the literature has revealed.

Gudzent claimed to have found a disappearance of uric acid from the blood after inhalation of air containing two to four Mache units of radium per hour. He thought it also caused an increase in the solubility of sodium urate. Kerb and Lazarus found that radium emanations had no effect on the solubility of sodium urate. *Kehrer* claimed that radium emanation caused a mobilization of uric acid in the body. *Knaffl-Lenz* and *Wiechowski* found no increase in the solubility or decomposition of sodium urate by radium emanation. *Fine* and *Chace* found that radium given as the bromide and inhalation of the emanation in strength as high as 100 Mache units per litre for long periods of time failed to show any influence upon the uric acid concentration of the blood and produced no definitely increased output of uric acid in the urine. *Skorczewski* claimed to have found that radium therapy in the gouty leads to an increased output of nitrogen, uric acid and neutral and oxidized sulphur.

*Nakanami* found an increased nitrogen excretion in cases of uterine cancer during radium therapy. This excretion was increased in direct proportion to the general improvement obtained.

*Wilke* and *Krieg* found that the ingestion of radio active water produced an increased excretion of uric acid. *Kikkoji* found the same and in one of his cases observed an increase of ninety-five per cent.

*Mesernitzky* claims that the uric acid of the blood is decreased by radium emanation and that there is an increased excretion of uric acid in the urine.

Von Noorden and Falta have also reported increased excretion of uric acid in cases of gout under the influence of radium emanation. McCrudden and Sargent found no change in the uric acid of the blood in a case of gout receiving 20,000 Mache units of impregnated water. They could find no change in the rate of excretion of uric acid and total nitrogen, but they did find a slight increase in the creatinine excretion which persisted for a few days after the radium treatment. These observers and also Fine and Chace have studied the effect of radium emanation on cases of chronic arthritis. They all found that the amount of uric acid in the blood and also the rate of excretion were unaffected by the radium emanation. McCrudden found a slight increase in creatinine excretion.

Ordway, Tait and Knudson studied the influence upon metabolism of surface application of radium emanation upon a case of sarcoma and upon a case of carcinoma. In the case of sarcoma they obtained increases in the urinary volume, in total acidity, ammonia, total nitrogen, urea, uric acid, creatinine and phosphates. In the case of carcinoma there was no increase of the nitrogenous fractions or phosphates of the urine.

Luden has found that radium reduces the high cholesterol values found in the blood of carcinoma patients and draws attention to the fact that this may play an important part in the beneficial effect of radium therapy.

Fofanow claimed that inhalation of emanation of radium produced an increased elimination of uric acid and of purin bases; both endogenous and exogenous were effected. This is followed by a disappearance of urate deposits and tophi and in gout there occurs a disappearance of uric acid from the blood. Kaplan found that alkaline radium waters decreased the uric acid and increased the purin bases of the urine.

Silbergleit studied the influence of baths containing radium emanation on the gaseous exchange of normal men and his results were negative. Kikkoji found a distinct increase in the basal metabolism of normal men who received three doses of 330 Mache units per os. Bernstein determined the basal metabolism of several persons before and after a two hour interval in an emanatorium containing from 220 to 440 Mache units per litre of air. The results were not definite. Benczur and Fuchs found the respiratory quotient unaffected after the ingestion of radium

emanation water containing 300,000 to 400,000 Mache units. Murphy, Means and Aub studied the basal metabolism of a case of chronic lymphatic leukemia treated with radium. They found no abnormal respiratory quotients but a slight fall in the basal metabolism. Knudson and Erdos in a case of myelogenous leukemia treated by surface application of radium, observed in each of the three series of treatment marked changes in metabolism. The total nitrogen, urea, ammonia and phosphates were immediately increased and reached a maximum about seven days after each application. The uric acid reacted the same. There was no change in the uric acid of the blood. In another case of myelogenous leukemia, Ordway, Tait and Knudson obtained similar results. An examination of the blood for creatinine and non-protein nitrogen, before, during and right after radium treatment showed no change. Staehelin and Maase found that alkaline radium water diminished the gaseous metabolism in health but not in people subject to gout.

## II. EXPERIMENTAL.

### (a). Methods.

#### 1. Diet.

During the entire experiments the patients received daily the Folin diet, consisting of:

Whole milk .....	500 cc.
Cream .....	300 cc.
Eggs, whole .....	450 gm.
Malted milk .....	200 gm.
Sugar .....	20 gm.
Sodium chloride .....	6 gm.
Distilled water .....	2,100 cc.

This mixture was prepared fresh every day and portions taken out for analysis. The stools were marked off by means of carmine.

#### 2. Methods used in urine analysis.

The nitrogen was estimated by Kjeldahl method, the ammonia by Folin's, the total by Benedict's, total and ethereal sulphates by Folin's method. The inorganic sulphates by subtracting the ethereal sulphates from the total sulphates and the neutral sulphur by subtracting the total sulphate sulphur from the total sulphur. Urea was estimated by Benedict's method, total phosphorous by the Neumann method.

Creatin and creatinine by Folin's method, uric acid and purins by the Kruger-Schmidt method. Calcium and magnesium by McCrudden's



TABLE 1.  
The Urinary Nitrogen Partition

URINE																	
Day	Volume c.c.	Total nitro- gen gm.	Urea Nitrogen		Uric acid			Ammonia nitrogen		Amino-acid nitrogen		Creatinin			Undetermined nitrogen		Remarks
			gm.	Per cent of total nitro- gen	gm.	Nitro- gen	Per cent of total nitro- gen	gm.	Per cent of total nitro- gen	gm.	Nitro- gen	Per cent of total nitro- gen	gm.	Per cent of total nitro- gen			
1.	700	6.40	5.2	81.2	0.23	0.08	1.3	0.265	4.1	0.275	4.3	0.57	0.21	0.37	5.8	100 microgr <sup>ms</sup> of radium ele- ment intra- venously. 100 microgr <sup>ms</sup> of radium ele- ment intra- venously.	
2.	700	5.10	4.2	82.3	0.27	0.09	1.8	0.204	4.0	0.206	4.04	0.71	0.26	0.14	2.7		
3.	1000	7.84	6.4	81.6	0.25	0.08	1.02	0.29	3.9	0.172	2.2	0.81	0.29	0.59	7.5		
4.	1590	7.74	6.5	83.8	0.30	0.10	1.3	0.25	3.2	0.25	3.3	0.93	0.34	0.30	3.8		
5.	1540	7.33	6.2	84.6	0.27	0.09	1.2	0.29	4.0	0.31	4.2	0.93	0.34	0.10	1.4		
6.	1010	7.24	6.0	82.9	0.28	0.09	1.2	0.23	3.1	0.25	3.5	0.80	0.29	0.38	5.2		
7.	1400	6.34	5.2	82.0	0.22	0.07	1.1	0.27	4.2	0.26	4.1	0.56	0.26	0.34	5.3		
8.	1380	7.54	6.3	83.6	0.28	0.09	1.2	0.29	3.8	0.28	3.7	0.91	0.33	0.25	3.3		
9.	1240	7.82	6.7	85.7	0.34	0.11	1.4	0.26	3.3	0.23	2.9	0.93	0.34	0.18	2.3		
10.	1280	7.12	6.0	84.2	0.28	0.09	1.2	0.25	3.5	0.21	2.9	0.78	0.28	0.29	4.1		

TABLE 2.  
The Urinary Sulphur Partition.

URINE										REMARKS	
Day	Total sulphur		Total sulphate sulphur		Inorganic sulphate sulphur		Etheral sulphate sulphur		Neutral sulphur		
	gm.	Per cent of total sulphur	gm.	Per cent of total sulphur	gm.	Per cent of total sulphur	gm.	Per cent of total sulphur	gm.	Per cent of total sulphur	
1.	0.57	87.7	0.50	87.7	0.48	84.2	0.02	3.5	0.07	12.3	100 micrograms of radium element intravenously.
2.	0.48	85.4	0.41	85.4	0.35	72.9	0.06	12.5	0.07	14.6	
3.	0.80	70.0	0.56	70.0	0.51	62.5	0.05	7.5	0.24	30.0	
4.	0.96	63.5	0.61	63.5	0.54	56.2	0.07	7.3	0.35	36.4	
5.	0.84	70.2	0.59	70.2	0.55	65.4	0.04	4.8	0.25	29.7	
6.	0.76	90.8	0.69	90.8	0.57	75.0	0.12	15.6	0.07	9.2	
7.	0.86	69.7	0.60	69.7	0.54	62.8	0.06	6.9	0.26	30.2	
8.	0.96	65.3	0.64	65.3	0.54	56.2	0.10	9.1	0.32	33.3	
9.	0.94	65.9	0.62	65.9	0.52	55.3	0.10	10.6	0.32	34.1	
10.	0.62	90.0	0.558	90.0	0.49	79.0	0.068	11.0	0.062	10.0	

method. Acidity by Folin's method. Amino-acid nitrogen by the Benedict-Murlin method. The undetermined nitrogen by subtracting the other nitrogen containing partitions from the total urinary nitrogen.

3. Methods used in analysis of food and feces.

Nitrogen by Kjeldahl, sulphur by Wolff and Osterberg modification of Benedict method. Calcium and magnesium by McCrudden's method and phosphorous by Neumann method.

(b). Influence of intravenous injections of radium salts on the urinary nitrogen and sulphur partition in a case of rheumatoid arthritis.

The patient, male, age 55, on whom this study was carried out was the subject of a severe case of rheumatoid arthritis of many years standing. The following tables, No. I and No. II, show the results obtained in this study:

*Discussion of Tables No. I and No. II.*

Table No. I demonstrates that following the intravenous injections of 100 micrograms of radium element the urinary nitrogen shows a marked increase on both occasions. This increase persisted for about three days. The proportions of urinary nitrogen as urea-nitrogen, uric acid-nitrogen, ammonia-nitrogen, amino-acid nitrogen, creatin-nitrogen and undetermined-nitrogen remained about the same following the injections. The uric acid in grams and uric acid-nitrogen, both of which are of special interest, also show the non-effect of the radium on their excretion in the urine.

Table No. II shows that following the intravenous injection of 100 micrograms of radium element there was produced a marked increase on both occasions in the total sulphur of the urine, and this increase persisted for three days. On the urinary sulphur partition, it may be noted that a marked increase in the neutral sulphur of the urine was produced by the radium. This effect continued for about three days following the injection. This finding of effects ceasing after three days following the injection may be considered as an indication for giving the radium about every fourth day, as by this method one would be renewing the effect that it has on the metabolism.

It will be recalled that the urinary sulphur is made up of sulphates, both inorganic and ethereal and certain less highly oxidized compounds which are called, following Salkowski's suggestion, "Neutral sulphur." The neutral sulphur compounds are many and include the following: Uroferric acid, uroproteic acid, oxyproteic acid, urochrome, thiocyanic acid and its salts, cystin

and similar substances, taurin and taurincarbamic acid, methyl mercaptan, ethyl sulphid, thiosulphuric acid and sulphuric acid.

This effect of intravenous injection of radium producing such a marked increase in the excretion of the urinary neutral sulphur is of great interest. It will be recalled that Folin claims that the truest index to the endogenous or cellular metabolism is represented by the urinary uric acid, creatinin and neutral sulphur. As we have found that the intravenous injection of radium in dosage of 100 micrograms has no effect on the amount of creatinin and uric acid excreted, it must be that the radium influences some part of the endogenous metabolism related to the neutral sulphur but not implicating the other two constituents. It may be that the radium affects the intracellular oxidation, thereby increasing the amount of neutral or unoxidized sulphur.

(b) Influence of *local* applications of radium on the metabolism of a patient with carcinoma.

The patient on whom this study was carried out had a rapidly growing carcinoma of the jaw. Throughout the experiment he was placed on the Folin diet, the plan of the experiment and the methods used for the estimation of various substances in the food and excreta were the same as those already described.

The container containing the radium was inserted, after an incision, deep into the cancerous mass, and was used for the following time and dosage:

Date	Amount of radium element	Time of exposure
12-30-13	16 milligrams	3 hours
12-31-13	" "	" "
1-1-14	" "	" "
1-2-14	" "	" "
1-3-14	70 "	1½ "
1-4-14	" "	3 "
1-5-14	" "	" "
1-6-14	" "	" "

It may be noted that the radium was applied on four different days previous to the start of the metabolism study, because it was thought by this procedure any effects produced would be more marked, on account of this previous dosage.

The following tables, No. III, IV, V, and VI, contain the results obtained in this study.

#### *Discussion of Table No. III.*

During the four days of metabolism study there was a nitrogen retention of 1.4 gram. The urea-nitrogen, ammonia-nitrogen,



Table No. III. The Nitrogen Metabolism and Urinary Nitrogen Partition

Date	URINE										URINE						FECES		NITROGEN																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																												
	Amt. c.c.	Total nitro- gen	Urea- nitrogen		Ammonia nitrogen		Creatinin			Uric acid			Purin- nitrogen		Acid- ity in	Amino-acid nitrogen		Undetermined nitrogen		Nitrogen		In- take	Bal- ance																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																								
			gm.	Per cent of total nitro- gen	gm.	Per cent of total nitro- gen	Nitro- gen	Per cent of total nitro- gen	gm.	Per cent of total nitro- gen	Per cent of total nitro- gen	Per cent of total nitro- gen	gm.	Per cent of total nitro- gen		gm.	Per cent of total nitro- gen	gm.	Per cent of total nitro- gen																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																												
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TABLE IV.  
The Sulphur Metabolism and Urinary Sulphur Partition

URINE														FECES	SULPHUR	
Date	Total sulphur gm.	Total Sulphate sulphur		Ethereal sulphate sulphur		Inorganic sulphate sulphur		Neutral Sulphur		Sulphur	Intake	Balance				
		gm.	Per cent of total sulphur	gm.	Per cent of total sulphur	gm.	Per cent of total sulphur									
1914										gm.	gm.	gm.				
1-3	1.33	1.27	95.5	0.21	15.8	1.06	79.7	0.06	4.5	0.45	1.88	+0.48				
1-4	1.35	1.23	91.1	0.19	14.0	1.04	77.1	0.12	8.9	0.45	1.80	-----				
1-5	1.32	1.20	90.9	0.18	13.6	1.02	77.3	0.12	9.1	0.45	1.79	+0.02				
1-6	1.36	1.24	91.1	0.19	13.8	1.05	77.3	0.08	8.9	0.45	1.80	0.01				

TABLE V.  
The Calcium, Magnesium, and Phosphorus Metabolism

	URINE			FECES			INTAKE			BALANCE		
	Calcium oxide gm.	Magnesium oxide gm.	Phosphorus gm.	Calcium oxide gm.	Magnesium oxide gm.	Phosphorus gm.	Calcium oxide gm.	Magnesium oxide gm.	Phosphorus gm.	Calcium oxide gm.	Magnesium oxide gm.	Phosphorus gm.
Date 1914												
1-3	1.17	0.13	1.72	1.56	0.42	0.90	2.8	0.62	2.92	†0.07	†0.07	†0.30
1-4	0.98	0.11	1.75	1.56	0.42	0.90	2.6	0.65	2.85	†0.06	†0.12	†0.20
1-5	0.96	0.14	1.70	1.56	0.42	0.90	2.3	0.60	2.80	0.06	†0.04	†0.20
1-6	0.92	0.14	1.65	1.56	0.42	0.90	2.4	0.62	2.85	†0.08	†0.06	†0.10
										†0.27	†0.29	†0.80

creatinin, uric acid, purin-nitrogen, amino acid-nitrogen and undetermined nitrogen are perfectly normal in amount and in percentage of the total nitrogen. The feces-nitrogen in per cent. of the total nitrogen ingested is normal. The acidity of the urine is also normal in amount.

#### *Discussion of Table No. IV.*

During the four days study there was a sulphur retention of 0.49 grams. The total sulphate-sulphur, ethereal-sulphate-sulphur, inorganic sulphate-sulphur and neutral sulphur is normal in character.

It is of great interest to note that the local application of radium in a case of cancer, did not affect the neutral sulphur, as does the intravenous injection of radium. This may be due to the fact that following the intravenous injection of radium the endogenous cellular metabolism is altered, possibly the oxidation mechanism leading to an increased neutral sulphur formation and excretion, while with local application of radium for the dosage and time exposure as used in this case, we only are dealing with the local effects of radium on the cells subject to its radiation, with a little deep absorption of same, and hence, none of the systemic effects of the radium are noted, that is as regards its effect on the nitrogen metabolism and sulphur metabolism.

#### *Discussion of Table No. V.*

During the four days study there was a retention of 0.27 gram calcium oxide, 0.29 gram magnesium oxide and 0.80 gram of phosphorus. The percentage excretion of calcium, magnesium and phosphorus in the urine and feces is normal.

TABLE VI.

Percentage excretion of calcium magnesium and phosphorus in the urine and feces.

Date 1914	URINE			FECES		
	Ca O %	Mg. O %	P %	Ca. O %	Mg. O %	P. %
1-3	42.9	23.6	65.6	57.1	76.4	34.4
1-4	38.5	20.7	66.1	61.5	79.3	33.9
1-5	38.1	25.0	65.4	61.9	75.0	34.6
1-6	37.1	25.0	64.7	62.9	75.0	35.3



### III. CONCLUSION.

1. The *intravenous injection* of 100 micrograms of radium element produced an increase in the amount of nitrogen excreted in the urine. No constant effect was noted on the percentage excretion of urea-nitrogen, ammonia-nitrogen, uric acid-nitrogen, creatinin-nitrogen, amino-acid nitrogen and undetermined nitrogen.

2. The injection also caused a marked increase in the amount of total urinary sulphur and in the amount of neutral sulphur excreted.

3. The effect of intravenous injection of 100 micrograms of radium on the nitrogen and sulphur metabolism lasted for about three days following the injection.

4. It is thought that the increased neutral sulphur excretion is due to the effect of the radium on the intracellular oxidation.

5. The *local application* of 16 milligrams of radium for 4 days and 70 milligrams of radium for 4 days was studied in a case of carcinoma as regards its effects on metabolism.

6. During the four days of this metabolism study there was a nitrogen retention of 1.4 gram. The urea-nitrogen, ammonia-nitrogen, creatinin, uric acid, purin-nitrogen, amino-acid nitrogen, undetermined nitrogen are normal in amount and in percentage of the total nitrogen.

7. The acidity of the urine was normal in amount.

8. During the four days there was a sulphur retention of 0.49 gram. The total sulphate sulphur, ethereal sulphate-sulphur, inorganic sulphate-sulphur and neutral sulphur are normal in character.

9. There was a retention of 0.27 gram calcium oxide, 0.29 gram magnesium oxide and 0.80 gram of phosphorus.

10. The percentage excretion of calcium, magnesium and phosphorus in the urine and feces is normal.

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# THE ISOLATION OF A HYPOGLYCEMIA-PRODUCING PRINCIPLE FROM VEGETABLES AND THE NATURE OF THE ACTION OF VEGETABLE EXTRACTS ON THE BLOOD SUGAR OF NORMAL RABBITS\*

By

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Following closely upon the discovery of insulin, many investigators tried to find sources of supply other than the mammalian pancreas. Thus, Collip<sup>1</sup> demonstrated an insulin-like substance in clam tissue, while Macleod<sup>2</sup> succeeded in preparing insulin from the principal islets of certain bony fishes.

In line with the theory that insulin, or a hormone similar to insulin, might be present wherever glucose is metabolized, considerable research has been carried on in an effort to extract the hypoglycemia-producing hormone from vegetable sources. The results obtained were sufficiently encouraging to justify a continuation of the work in the hope of finding a more universally distributed and cheaper source of supply than the pancreas.

As far back as 1914, Funk and v. Schoenborn<sup>3</sup> observed that the injection of vitamine B, prepared from yeast, into pigeons kept on a vitamine-free diet, resulted in a drop in blood sugar from 0.29 to 0.19 per cent. This finding takes on added interest in view of the recent experiments establishing the point that there is present in yeast, plants and vegetables a substance which, upon injection into normal rabbits, causes a fall in blood sugar.

The work of Boruttau<sup>4</sup> pointed to the conclusion that the cortical layer of oats contains a specific anti-diabetic substance. Warner, Dixon and Dixon<sup>5</sup> obtained a substance from yeast which lowered the blood sugar and diminished the sugar output of diabetic dogs. Winter and Smith<sup>6</sup> were able to show conclusively the presence of an insulin-like substance in yeast. A somewhat similar observation was made by Fetzner.<sup>7</sup> Best and Scott,<sup>8</sup> working with extracts of potatoes, rice, wheat, beet roots and celery, demonstrated a substance which, when injected into normal rabbits, caused a marked decrease in blood sugar. Thalhimer and Perry<sup>9</sup> also noted a decrease in blood sugar after the injection of raw potato juice.

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\* Read in part before the Medicinal Products and Biological Divisions at the Milwaukee meeting of the American Chemical Society, September 13, 1923, and in part before the Society for Experimental Biology and Medicine, October 17, 1923.

TABLE I.

Blood sugar of normal rabbits after injection of various extracts of cabbage—"delayed and prolonged" action of crude extracts.

Rabbit		Preparation Injected	Cabbage Equiv- alent	Time After Injec- tion	Blood Sugar	Change in Sugar Content	Remarks
No.	Weight						
21	gm. 1410	<i>C-1a</i> Precipitate from 80% alcoholic solution.	gm. 200	hrs. 0	% 0.138	+ 7.2	
				2	0.136		
				4	0.148		
				6	0.147		
30	1875	<i>C-1b</i> Same as C-1a.	600	0	0.132	+51.3	
				2	0.164		
				4	0.162		
				6	0.158		
35	1640	<i>C-1c</i> Same as C-1a	527	0	0.131	+19.1 —15.3 —16.8 —32.1 —43.5 —37.4	Food withheld during entire period.
				2	0.156		
				14	0.111		
				17	0.109		
				20	0.089		
				47½	0.074		
				50½	0.082		
36	2365	<i>C-1d</i> Same as C-1a	760	0	0.127	+22.0	Food withheld during entire period.
				2	0.155		
				14	0.127		
				17	0.135		
				20	0.124		
				47½	0.120		
				50½	0.129		

Funk and Corbitt<sup>10</sup> found that the injection of sterilized fresh whole yeast cells resulted in a decrease in blood sugar amounting to 40 per cent. within 4½ hours. In another experiment an 80 per cent. alcoholic extract produced an appreciable hypoglycemia.

On the other hand, Funk and Corbitt (unpublished), as well as Collip,<sup>11</sup> observed from their experiments with yeast and various vegetable extracts that there was usually a preliminary hyperglycemia and that the blood sugar-reducing effect did not manifest itself until some time after the injection. They found also that the hypoglycemia was more prolonged than was the case with insulin.

Because of this "delayed and prolonged" action, the above investigators conclude that the blood sugar-reducing substance present in vegetables is a new hormone. Funk and Corbitt<sup>10</sup> thought this might be a "pre-insulin," while Collip<sup>11</sup> named the substance "Glucokinin."

Having in mind the possible influence of the blood sugar-increasing substance in masking the true action of the hypoglycemia-producing factor, we attempted to effect a separation of



these two principles present in vegetable extracts. Cabbage, celery, spinach, lettuce, red beets and carrots—all of which were obtained freshly cut—were used in this investigation.

TABLE II.

Blood sugar of normal rabbits after injection of suprarenin (synthetic) solutions—illustrating “delayed and prolonged” action.

Rabbit		Fraction Injected	L-Suprarenin Base; cc. of 1: 1000 per kg.	Time After Injection	Blood Sugar	Change in Sugar Content	Remarks
No.	Weight						
21	gm. 1680	l—Suprarenin bitartrate.	1	hrs.	%	%	
				0	0.177	+119.0	
				2	0.388		
				4	0.343		
				6	0.288	—27.0	
				23	0.125		
				26	0.133		
				30	0.143		
26	1330	l—Suprarenin bitartrate.	1	0	0.115	—58.2	Convulsions at 22 hrs. Rabbit fed but died during night.
				15	0.066		
				18	0.048		
				22	0.059		
34	1680	l—Suprarenin bitartrate.	1	0	0.135	+213.0	
				2	0.423		
				14	0.142		
				16	0.152		
				18	0.151		
				20½	0.159		
35	1635	r—Suprarenin hydrochloride.	1	0	0.124	+142.0	
				2	0.300		
				4	0.294		
				6	0.244		
				9	0.138		
				11½	0.106		
				14	0.112	—19.4	
				25	0.101		

Our preliminary findings were substantially like those noted by the above investigators. Subsequent experiments, however, tended to show that the blood sugar-reducing substance obtained from vegetable sources, if freed from the hyperglycemia-producing principle, behaves in a manner similar to that exhibited by insulin.

*Method of preparing hypoglycemia-producing principle from vegetables\**

The fresh vegetable is ground very fine and extracted for a number of hours with sufficient 95 per cent. alcohol to make a 70 per cent. alcoholic solution. After standing in a cool place over night, the mixture is filtered and the residue pressed out. The combined filtrates are evaporated *in vacuo* to about 75 cc. for every kilo of vegetable used. The chlorophyll, which separates out, is removed with ether and the solution evaporated down to 60 cc. This is then made up to 80 per cent. with 95 per cent. alcohol and allowed to stand in a cool place over night.

TABLE III.

Blood sugar of normal rabbits after injection of various fractions obtained from cabbage—showing action of blood sugar-increasing substance.

Rabbit		Fraction Injected	Cabbage Equiv- alent	Time After Injection	Blood Sugar	Change in Sugar Content	Remarks
No.	Weight						
13	gm. 1805	A-1 80% alcoholic extract	gm. 109	hrs. 0	% 0.133	%    +14.3	
				2	0.140		
				4	0.144		
				6	0.142		
				24	0.152		
16	1450	A-3 Same as A-1, after treating with char- coal.	109	0	0.140		
				2	0.146		
				4	0.139		
				6	0.136		
				24	0.135		
26	1610	A-4 Precipitate from 93% alcoholic solution made from 80% alcoholic extract which had been treated with char- coal.	512	0	0.124	—9.7	Small variations up or down are regarded as insignificant.
				2½	0.112		
				4½	0.130		
				6	0.131		
				23¼	0.122		
16	1385	A-5 Filtrate from A-4.	512	0	0.127	+249.0 +480.0	Rabbit died at 4¼ hours. Last bleeding from heart.
				2	0.320		
				4¼	0.736		

The clear supernatant liquid is decanted, leaving a brown syrupy mass which contains the bulk of the blood sugar-increasing substance. The 80 per cent. solution is now made up to 93 per cent., with 95 per cent. alcohol and allowed to stand in a cool place over night. The almost clear supernatant liquid is poured off, the grayish-white precipitate is centrifuged, washed with alcohol and ether and dried. (Beets and carrots alone failed to give a precipitate.)

\* We take this opportunity to express our appreciation of the assistance rendered by Mr. Irving Cole in the preparation of the vegetable extracts.

In some instances this precipitate, when dissolved in water and injected, produced a marked hypoglycemia. In most cases, however, a further purification is necessary. For this purpose the precipitate is dissolved in water and treated with a saturated aqueous solution of dinitrosalicylic or picric acid. The precipitate obtained is decomposed with hydrochloric acid-alcohol and the alcoholic solution treated with ether to obtain the hydrochloride which, upon injection, displayed a pronounced hypoglycemia-producing effect.

TABLE IV.

Blood sugar of normal rabbits after injection of various extracts of cabbage—showing effect of removing the blood sugar-increasing substance.

Rabbit		Preparation Injected	Cabbage Equivalent	Time After Injection	Blood Sugar	Change in Sugar Content	Remarks
No.	Weight						
26	gm. 1340	<i>C-3a</i> Precipitate from 93% alcoholic solution.	gm. 1300	hrs. 0 2 3 $\frac{3}{4}$ 4	% 0.137 0.054 0.043	%  —68.7	Convulsions at 3 hours. 2 gm. glucose followed by recovery.
18	2575	<i>C-3b</i> Same as C-3a.	1700	0 1 $\frac{2}{3}$ 1 $\frac{5}{6}$ 4	0.144 0.028 0.120	—80.7	Convulsions at 1 $\frac{2}{3}$ hours. 2 gm. glucose intraperitoneally. Recovery.
29	1855	<i>C-4</i> Precipitate from 93% alcoholic solution, after previous extraction of 80% alcoholic solution with charcoal.	1584	0 2 4 6	0.126 0.123 0.135 0.133		
13	1910	<i>C-5a</i> Precipitate resulting from the purification of some of C-3a with dinitrosalicylic acid.	1000	0 2 4 6	0.143 0.088 0.127 0.145	—38.5	No convulsions.
28	1685	<i>C-5b</i> Filtrate from C-5a.	1000	0 2 4 6	0.136 0.136 0.138 0.130		

By the above method, one kilo of vegetable yields approximately 0.100 gm. of the crude grayish-white precipitate. From this, in turn, it is possible to obtain about 0.010 gm. of the very potent hydrochloride.\* The

\* Immediately after completing our work and preparing it for presentation at the September meeting of the American Chemical Society, we received a copy of the *Biochemical Journal*, from which we learned that Dudley<sup>9</sup> had used practically the same procedure in preparing and purifying insulin from pancreas.



purified substance gives positive nitrogen, sulphur, Molisch and biuret tests. The Millon test is negative, as is also that for phosphorus.

An active insulin-like substance was obtained as well by the aqueous acid extraction described by Murlin,<sup>13</sup> supplemented by the further purification outlined above.

TABLE V.

Blood sugar of normal rabbits after injection of various fractions obtained from cabbage—showing effect of elimination of blood sugar-increasing substance.

Rabbit		Fraction Injected	Cabbage Equiv- alent	Time After Injec- tion	Blood Sugar	Change in Sugar Content	Remarks
No.	Weight						
27	gm. 1645	<i>A-8a</i> Precipitate from 93% alcoholic solution.	gm. 908	hrs. 0 2 6	% 0.134 0.031	% —77.0	Convulsions at 2 hours. Intraperitoneal injection of 2 gm. glucose which was insufficient. Rabbit found dead.
28	1950	<i>A-8b</i> Same as A-8a.	454	0 3 4½ 6½ 23½	0.139 0.118 0.130 0.134 0.133	—15.1	
29	1745	<i>A-9</i> Filtrate from A-8a.	572	0 2 4 6 23½	0.128 0.149 0.152 0.164 0.148	+28.1	
21	1560	<i>A-12a</i> Precipitate resulting from purification of some of A-8a with picric acid.	1362	0 2 2¾ 3¼ 5¼ 5½	0.135 0.057 0.062 0.045	—57.8 —70.3	Convulsions at 1½ hours; again at 3½ hours. 2 gm. glucose intraperitoneally after bleeding. 2 gm. glucose subcutaneously. Recovery.
23	1375	<i>A-12b</i> Filtrate from A-12a.	1362	0 2 4 6 24½	0.136 0.117 0.137 0.146 0.132	—14.0	Blood clotted in pipette on second bleeding.
24	1305	<i>B-2a</i> Crude precipitate obtained by aqueous acid extraction.	1181	0 1 2½	0.135 0.053 0.045	—70.3	Convulsions 1 hour died 2½ hours.

The hypoglycemia-producing principle from vegetables may be adsorbed by charcoal, as was pointed out in work on pancreas extracts by Best and Macleod,<sup>14</sup> and by Murlin, Clough, Gibbs and Stokes.<sup>15</sup> The hypoglycemia-producing substance may be extracted from the charcoal (norit) with

glacial acetic acid. The acid may then be evaporated off *in vacuo*, the residue taken up in water and the active substance precipitated out with dinitrosalicylic or picric acid. The active principle may also be precipitated from the glacial acetic acid directly by ether. This has recently been confirmed by Widmark<sup>16</sup> in his work on the solubility of insulin. Further confirmation is found in the experiments of Moloney and Findlay,<sup>17</sup> who use glacial acetic acid to dissolve a crude insulin precipitate. From the acid, the insulin is then precipitated with ether, or on adding sufficient ether and water, the insulin is found in the watery layer.

TABLE VI.

Blood sugar of normal rabbits after injection of various extracts of cabbage—showing that hypoglycemia-producing principle may be adsorbed by charcoal.

Rabbit		Preparation Injected	Cabbage Equivalent	Time After Injection	Blood Sugar	Change in Sugar Content	Remarks
No.	Weight						
18	gm. 2360	Z-6a 80% alcoholic solution treated with charcoal, latter extracted with glacial acetic acid on water bath.	gm. 292	hrs. 0 2 4½ 6 23½	% 0.125 0.128 0.126 0.122 0.136	%	
13	1805	Z-6b Above charcoal again extracted with boiling glacial acetic acid.	292	0 2 4½ 6 23½	0.142 0.122 0.130 0.139 0.136	—14.1	
28	1835	C-2a 80% alcoholic solution treated with charcoal; latter boiled with glacial acetic acid.	1056	0 2 2½ 3 3½ 6½	0.115 0.045 0.042 0.042 0.059	—61.0 —63.5	Convulsions within 1½ hours. 2 gm. glucose intraperitoneally. 2 gm. glucose-subcutaneously. Rabbit recovered.
30	1950	C-2b Same as C-2a.	528	0 2¼ 4½ 6	0.138 0.143 0.136 0.149		
25	1670	A-2a 80% alcoholic extract treated with charcoal; latter extracted with glacial acetic acid on water bath.	436	0 2½ 4½ 6 23½	0.135 0.140 0.135 0.129 0.133		
19	2340	A-2b Same as A-2a, using boiling glacial acetic acid.	654	0 2 3½	0.102 0.055 0.044	—56.8	Convulsions at 3 hours followed by death at 4½ hours.

*Experimental*

The rabbits were fed on a diet of oats, carrots, cabbage and water, and kept in metal cages so constructed that both urine and feces were voided beyond the reach of the animals. They were deprived of food 16 hours before being injected with the substance under investigation, and throughout the course of the experiment. Observations were made over periods ranging from 2 to 54 hours. Injections were made subcutaneously, the average quantity of liquid injected being about 10 cc.

Sugar was estimated by the method of MacLean,<sup>18</sup> as modified by Hastings and Hopping.<sup>19</sup>

*Results*

Owing to the accumulation of a large mass of data, only a limited number of representative experiments were chosen to illustrate our findings.

Table I shows the effect of injecting the syrupy precipitate obtained from the 80 per cent. alcoholic solution. Where the observations extended over a period of 50½ hours there was, in one case, a rise in blood sugar, while in the other, the rise was followed by a fall below normal. In two other experiments, where observations were made for only 6 hours, only a rise in blood-sugar was noted.

TABLE VII.

Blood sugar of normal rabbits after injection of extract of celery (stalks and leaves).

Rabbit		Preparation Injected	Celery Equivalent	Time After Injection	Blood Sugar	Change in Sugar Content	Remarks
No.	Weight						
35	gm. 1625	Precipitate from 93% alcoholic solution.	gm. 996	hrs. 0	% 0.118	% 	
				2	0.113		
				4	0.124		
				6	0.119		
36	2465	Same as above.	1992	0	0.127		
				2	0.133		
				4	0.128		
				6	0.133		
13	2130	Precipitate from 93% alcoholic solution purified with picric acid.	1992	0	0.152	—56.0	No convulsions.
				2	0.067		
				4	0.125		
				6	0.131		



That a somewhat similar effect—"delayed and prolonged action"—could be obtained with suprarenin (synthetic) is evident from Table II. Here we see, in one rabbit, a rise in blood sugar, while in the three remaining animals there was a preliminary increase followed by a fall below normal. A 2 hour observation was not made in the case of rabbit No. 26, thereby missing the usual rise in blood sugar; however, at 18 hours the blood sugar had decreased from 0.115 to 0.048 per cent. At 22 hours the animal was seized with convulsions, and died during the night.

TABLE VIII.

Blood sugar of normal rabbits injected with various spinach preparations.

Rabbit		Preparation Injected	Spinach Equivalent	Time After Injection	Blood Sugar	Change in Sugar Content	Remarks
No.	Weight						
18	gm. 2750	S-1 Precipitate from 93% alcoholic solution. (Neutral alcoholic extract.)	gm. 838	hrs. 0	% 0.137	—13.9	
				2	0.118		
				4	0.136		
				6	0.138		
37	1595	S-2 Same as S-1.	1520	0	0.122	+48.3	
				2 $\frac{1}{3}$	0.120		
				4	0.134		
				6 $\frac{3}{4}$	0.181		
13	2090	S-3 Precipitate from 93% alcoholic solution. (Acid alcoholic ex- tract.)	1257	0	0.153	+62.2	
				2 $\frac{1}{4}$	0.191		
				4 $\frac{1}{4}$	0.214		
				6	0.248		
33	1350	S-4 Same as S-3, purified with picric acid.	1257	0	0.117	—83.0	No sugar found in 1 cc. blood, at 2 hours. 5 cc. blood from heart. Convulsions; animal died; bled from heart immediately after death, at 4 $\frac{1}{2}$ hours.
				2			
				3	0.033		
				4 $\frac{1}{2}$	0.020		

The outstanding feature of Table III is the fact that if an 80 per cent. alcoholic solution is first shaken with charcoal and then made up to 93 per cent., the resultant precipitate shows only a very slight blood sugar-reducing action. As will be seen later, the charcoal has adsorbed the hypoglycemia-producing principle. Rabbits No. 13 and No. 16 showed no conclusive results, presumably because of insufficient material injected.

An examination of Tables IV and V shows that a powerful hypoglycemia-producing principle is obtained when the blood sugar-increasing factor has been removed. It is further evident that the highly purified active principle may be precipitated completely from a solution of the crude 93 per cent. precipitate, by dinitrosalicylic or picric acid. The results with rabbit No. 24 show that the active principle may also be obtained by means of an aqueous acid extraction.

The blood sugar reduction noted in rabbit No. 23 (Table V) is undoubtedly due to the fact that some of the blood clotted in the pipette. In other cases, similar filtrates proved inactive.

Table VI illustrates conclusively the ability of charcoal (norit) to adsorb the hypoglycemia-producing substance, and its subsequent extraction with boiling glacial acetic acid. It is not enough merely to have the acid hot.

In Tables VII and VIII, we see that the hypoglycemia-producing principle was obtained only upon purification with picric acid.

TABLE IX.

Blood sugar of normal rabbits after injection of old and fresh lettuce preparations.

Rabbit		Preparation Injected	Lettuce Equivalent	Time After Injection	Blood Sugar	Change in Sugar Content	Remarks
No.	Weight						
13	gm. 2075	<i>L-1</i> Precipitate from 93% alcoholic solution. (old lettuce.)	gm. 3050	hrs. 0	% 0.136	+5.9	Small variations up or down are regarded as insignificant.
				2	0.132		
				4	0.144		
				6	0.140		
32	1565	<i>L-2</i> Same as L-1.	2450	0	0.116	+15.5	Appreciable increase.
				2	0.124		
				4	0.134		
				6	0.123		
45	2225	<i>L-3</i> Same as L-1. (using fresh lettuce.)	2000	0	0.131	—14.5	Definite though small reduction.
				2	0.112		
				4	0.115		
				6	0.127		
47	2020	<i>L-4</i> Same as L-3.	2000	0	0.120	—14.2	Definite though small reduction.
				2	0.103		
				4	0.109		
				6	0.117		

Table IX indicates that only fresh tissue yields a blood sugar-reducing substance. Some reduction in blood-sugar is noted in rabbits No. 45 and 47. Unfortunately, there was not enough material available to effect a further purification by means of dinitrosalicylic or picric acid.

TABLE X.

Blood sugar of normal rabbits after injection of normal rabbit blood.

Rabbit		Preparation Injected	Amount Injected	Time After Injection	Blood Sugar	Change in Sugar Content	Remarks
No.	Weight						
39	gm. 1725	Oxalated blood from ear vein of rabbit No. 40.	6 cc.	hrs.	%	%	Small variations up or down are regarded as insignificant.
				0	0.137		
				2	0.143		
				4	0.139		
				6	0.137		
				23 $\frac{3}{4}$	0.141		
				27	0.126	—8	
				30	0.141		
				47 $\frac{3}{4}$	0.151	+10	
				50 $\frac{3}{4}$	0.144		
				54	0.140		
40	1735	Whole blood from rabbit No. 39.	6 cc.	0	0.134		From heart. " " " " " " " "
				21 $\frac{1}{4}$	0.140		
				41 $\frac{1}{2}$	0.141		
				61 $\frac{1}{2}$	0.140		
				24	0.117	—12.7	
				27	0.135		
				30	0.130		
				48	0.139		
				51	0.122		
				54	0.107	—20.1	
40	1385			7 days	0.146		Normal diet.
41	1790	Normal blood from rabbit No. 40.	6 cc.	0	0.113		Longer period of observation impossible.
				11 $\frac{1}{2}$	0.117		
				3	0.121		
				5	0.118		
				23	0.136	+20.4	

In order to satisfy ourselves as to the effect of injecting normal whole blood into a normal rabbit, the experiments described in Table X were undertaken. In one case only, rabbit No. 40, was a reduction in blood-sugar obtained. True, this is only one instance, but it shows that normal blood itself may produce a fall in blood sugar. This is not at all surprising when we consider that insulin has been found in blood. All this, of course, is not



intended to disprove Collip's<sup>11</sup> statement as to the animal passage of a hypoglycemia-producing principle.

### *Discussion*

From a survey of the accompanying data, it is obvious that vegetables contain a hyperglycemia—as well as a hypoglycemia-producing principle. If extracts containing both of these substances are injected, there is usually a preliminary rise in blood sugar, followed by a fall below normal, which becomes apparent after a lapse of about 14 hours, occasionally after 24 hours or even longer.

This type of action, so different from that of insulin, was taken as a basis for the postulation of the theory that a new hypoglycemia-producing hormone was involved. The existence of the blood sugar-increasing substance was recognized, but its possible inhibitory action upon the blood sugar-reducing principle was apparently neglected.

We felt that we could duplicate the “delayed and prolonged” effect manifested by crude vegetable extracts by the injection of any substance—for example, epinephrin—that would cause a preliminary rise in blood sugar. This actually proved to be the case, and taken together with the observation that the mere ingestion of substances, such as glucose, galactose, fructose or starch produces a rise in blood sugar, followed by a fall below normal, as was shown by Liefmann and Stern,<sup>20</sup> Frank,<sup>21</sup> McLean and de Wesselow,<sup>22</sup> and Foster,<sup>23</sup> the supports erected to sustain the theory of the existence of a new vegetable hypoglycemia-producing hormone become somewhat insecure.

That the blood sugar-increasing substance of vegetables finds its counterpart in pancreatic insulin is evident from a perusal of a number of publications on the subject.

Collip<sup>24</sup> obtained “delayed action” with certain pancreatic extracts. Fisher<sup>25</sup> isolated a substance from insulin which produced a rise in blood sugar. Kimball, Allen and Piper<sup>26</sup> made some interesting observations in this respect. In one instance, a precipitate from insulin, thrown down by  $(\text{NH}_4)_2 \text{SO}_4$ , showed a rise of 79 mg. against an original drop of 69 mg. Slight rises were noted in the case of the precipitate obtained with alcohol and in the filtrates when inorganic reagents were used, even

though dialyzed and though the evaporated solution showed no microscopic evidence of the crystals of the substance used.

Murlin, Clough, Gibbs and Stokes<sup>15</sup> found two substances present in extracts of pancreas; one lowers the blood sugar and D:N ratio and raises the R. Q.; the other raises the blood sugar, both of normal and depancreatized animals enormously, and possibly causes the abrupt fall in the R. Q. to the diabetic level after 3 to 5 hours. This abrupt fall was noted quite regularly and obviously could not be due to the exhaustion of insulin, since the quotient rises again later on. Evidently after the blood sugar-increasing substance had exerted its effect, the influence of the blood sugar-decreasing principle became apparent.

From the foregoing, it is obvious that the true evaluation of the hypoglycemia-producing effect of vegetable extracts was not possible, unless the blood sugar-increasing substance could be eliminated. When this was accomplished, the vegetable hypoglycemia-producing principle, when injected into normal rabbits, produced results similar to those following the administration of insulin.

It is to be noted that no attempt has been made as yet to establish any definite relationship between the size of the dose injected and the resultant hypoglycemia. Consequently, we cannot speak of the vegetable hypoglycemia-producing substance in terms of "rabbit units." We can merely point out that one dose gave a reduction in blood sugar, while another resulted, besides, in convulsions. We feel justified, however, in saying that the yields of vegetable blood sugar-reducing substance obtained thus far do not augur well for vegetables as a source of supply.

Regarding the possible identity of the vegetable hypoglycemia-producing principle with insulin, we are not prepared to express a definite opinion, since we do not know what insulin is.

Judging from the experiments of Funk and v. Schoenborn,<sup>3</sup> from the same general method of preparation, and from somewhat similar characteristics as regards solubility, stability, etc., the possibility suggests itself that there may be some relationship between insulin and vitamine B. Considerable work would, of course, be necessary to establish this particular point.

Experiments are now in progress with the idea of throwing some light on the nature of insulin.

### Summary

It is demonstrated that cabbage, celery, lettuce and spinach contain both hyperglycemia—and hypoglycemia-producing substances.

Red beets and carrots contain only the blood sugar-raising principle.

Crude vegetable extracts, when injected, may produce either a rise in blood sugar or a rise followed by a fall below the normal.

The same effect may be produced by the injection of l-suprarenin bitartrate (synthetic).

On fractionating certain crude vegetable extracts, it is possible to separate the hyperglycemia—from the hypoglycemia-producing principle.

The preliminary extraction and fractionation is effected with alcohol by means of which a crude grayish-white precipitate is obtained. The yield is about 0.100 gm. per kilo of vegetable. In a number of cases, this precipitate produced hypoglycemia.

A further fractionation is usually necessary, involving the use of dinitrosalicylic or picric acid.

The blood sugar-decreasing substance is found in the dinitrosalicylic or picric acid precipitate, the filtrate being inactive.

From the precipitate, the active substance may be obtained as a hydrochloride by decomposing with hydrochloric acid-alcohol and precipitating with ether. The yield is about 0.010 gm. per kilo of vegetable.

The purified active principle gives positive nitrogen, sulphur, Molisch and biuret tests. The Millon test is negative, as is also that for phosphorus.

The hypoglycemia-producing substance may also be obtained by means of aqueous acid extraction and subsequent purification with dinitrosalicylic or picric acid.

The blood sugar-reducing principle may be adsorbed by charcoal, from which it may be liberated by glacial acetic acid.

From the glacial acetic acid, the active principle may be precipitated directly with ether.

As an alternative procedure, the glacial acetic acid may be evaporated off *in vacuo* and the residue taken up in water, from which the active principle may be precipitated by dinitrosalicylic or picric acid.



The vegetable hypoglycemia-producing principle, when freed from the blood sugar-increasing substance and injected, gives results similar to those produced by insulin.

The "delayed and prolonged" action of vegetable extracts described by other investigators may be due to the counter-effect of the blood sugar-increasing substance.

It may be mentioned, in passing, that the mere ingestion of glucose, galactose, fructose or starch produces a rise in blood sugar, followed by a fall below normal.

It is possible to isolate from pancreatic insulin a fraction which produces a rise in blood sugar.

In one experiment, 6 cc. of whole blood of a normal rabbit injected into another normal rabbit resulted in a 20 per cent. decrease in blood sugar in 54 hours. Two other experiments gave doubtful results.

The yields of the vegetable hypoglycemia-producing substance obtained thus far are not sufficient to warrant the use of vegetables as a source of supply.

No attempt has been made as yet to establish a "rabbit unit" of the vegetable blood-sugar-reducing substance. However, in some cases the hypoglycemia was accompanied by convulsions, which could be relieved by glucose.

Based upon the same general method of preparation and upon other points of similarity, such as solubility, stability and adsorbability, there may be some relationship between insulin and vitamin B.

### *Conclusions*

The blood sugar-reducing substance present in various plants and vegetables, when freed from the blood sugar-increasing principle and injected into normal rabbits, produces a fall in blood sugar typical of that caused by an injection of insulin.

This observation necessarily militates against the theory of the existence of a new hypoglycemia-producing hormone in plant tissue, particularly in view of the fact that it is possible to duplicate the "delayed and prolonged" blood sugar-reducing action by the injection of a number of widely separated and unrelated substances.

Until we know what insulin is, we cannot say that the vegetable blood sugar-reducing principle is or is not identical with insulin, except as shown herewith.

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## RESPIRATORY METABOLISM AND BLOOD CHEMISTRY OF FILIPINOS.

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The few reports on any phase of metabolism in the tropics have been concerned chiefly with the question of urinary secretion. (McCay,<sup>1</sup> Aron,<sup>2</sup> Campbell,<sup>3</sup> Concepcion,<sup>4</sup> Read and Wang.<sup>5</sup>) The present article is a report of work on the basal metabolism and blood chemistry in Filipinos undertaken as part of a study of beriberi in the Philippine General Hospital, Manila. While no results were obtained characteristic of this disease, it is thought that the figures have value as negative evidence in beriberi and, with the normal controls used, may serve to indicate the levels of metabolism that obtain among natives in the tropics.

The patients studied fall into two main groups; those with the confirmed diagnosis of beriberi, and normal controls, the latter being patients convalescing from minor traumata or from surgical conditions which are known not to affect the basal metabolic rate or to show abnormal figures in blood chemistry. Included in the beriberi group is one nursing mother whose infant was admitted to hospital with acute infantile beriberi and who, herself, gave evidence of a peripheral neuritis. As shown by Table I and Table II both these groups were on substantially the same diet in regard to the intake of protein, fat and carbohydrate, the chief difference being the higher content of green foods rich in water soluble B vitamine in the diet given the beriberi patients.

TABLE I.

*Typical Diet Given Beriberi Patients.*

Breakfast	No.	Amount.
Egg, boiled or fried.....	1	40 gm.
Bread .....		100 “
Butter .....		8 “
Banana, orange or chico.....	1	
Coffee or cocoa, with cream and sugar.....		1 cup



## Lunch

Rice, boiled .....	210 gm.
Bread .....	50 "
Sprouted mongo beans cooked with tomatoes.....	120 "
Beef steak or stew .....	100 "
Banana .....	1
Lettuce and tomato salad.....	120 "
Tea, with cream and sugar.....	1 cup
Or milk .....	200 c. c.

## Dinner

Fish, baked or stewed.....	100 gm.
Beans, boiled with tomato and onion.....	120 "
Rice, boiled .....	200 "
Eggplant, baked .....	100 "
Tapioca pudding .....	120 "

Milk or lemonade between meals as desired.

Squash, cabbage, camote (sweet potato) tops, pechay ("greens" similar to spinach), cucumber and upo (white squash) are alternated with the mongo beans.

TABLE II.

*Typical Diet Given Surgical Convalescents.*

Breakfast	No.	Amount
Beef hash .....		100 gm.
Rice, boiled .....		240 "
Banana or chico .....	1	
Coffee, with cream and sugar.....		1 cup
Luncheon		
Rice, boiled .....		320 gm.
Vegetables as in beriberi diet.....		100 "
Beef, minced with tomato and onion.....		110 "
Fried saba (banana) .....		60 "
Tea, with sugar .....		1 cup
Dinner		
Fish, baked with tomato sauce.....		80 gm.
Rice, boiled .....		320 "
Bread pudding .....		120 "
Coffee, with cream and sugar.....		1 cup

In the main the technic of Boothby and Sandiford<sup>6</sup> was followed. The Bailey<sup>7</sup> modification of the Tissot gasometer was used with valves made in the laboratory from gas mask butterfly valves. For connecting to the patient the mouth piece and nose clip from the RFK gas mask were used.

Somewhat to our surprise no difficulty was realized in completely closing the nostrils, which, in Filipinos, are apt to be broad and flat, by this simple clip. A nurse who spoke the dialect of the patient accompanied him to the metabolism room in every instance and remained with him throughout the test. This did much to reassure the patient. Without exception their behavior was all that could be desired; they co-operated well in maintaining closure of the lips about the mouth piece and obeyed all requests to remain absolutely quiet. The expired air was sampled over a mixture of equal parts of glycerine and a saturated solution of sodium chloride (McCann<sup>8</sup>) and analyzed in a Henderson gas analysis apparatus calibrated with mercury. Analyses were made in duplicate and only accepted if agreeing to 0.04 per cent. for carbon dioxide and 0.06 per cent. for oxygen.

For the calculation of the non-protein respiratory quotient and the partition of the metabolism between protein, fat and carbohydrate combustion, the urine was collected for periods including the whole metabolism run, usually about an hour. For this the patient was made to void when first reporting, before the test proper started, this time being noted and care being taken that he emptied his bladder completely. This first specimen was discarded, serving only to mark the time of the beginning of secretion of the sample. After the metabolism run was completed the patient again emptied his bladder completely, voiding directly into a wide mouth bottle, the time of voiding being noted. The specimen was rinsed into a volumetric flask of convenient size, made up to the mark with distilled water, and the nitrogen determined by the Folin micro-Kjeldahl method. This nitrogen output was computed to an hourly basis.

Blood analysis was done by the Folin and Wu<sup>9</sup> system except the determination of uric acid which was done by the new method of Benedict.<sup>10</sup> All bloods were taken in the morning before breakfast.

The case records of the patients studied are as follows:

#### GROUP I. BERIBERI PATIENTS.

##### R. I. 102418.

Filipina, 35 years old, lavendera. Admitted to hospital October 17, 1922, in last month of pregnancy.

Had smallpox, measles and chickenpox as child, malaria and dysentery in adolescence.

Five previous pregnancies. During the last months of all of these she was troubled with numbness of the legs which increased after delivery so that she would be unable to walk. Her first baby died at six weeks, the others are living. All but the first were given tikitiki extract throughout the period of nursing as was the mother.

*Present pregnancy:* For the first time the patient has experienced dimness of vision and dryness of the throat as well as the customary numbness of legs. Baby born November 11, 1922 in normal labor.

*Present condition:* Up and about but easily fatigued. There is numbness over the entire body, especially marked around the mouth, in the

lower extremities and in the scalp. The second pulmonic sound is accentuated. Knee jerks are absent. Calf muscles are slightly tender. There is slight oedema of the legs.

C. P. 103692.

Mother of a female infant, 28 days old, admitted to hospital with convulsions and cyanosis.

Third pregnancy. Has had slight numbness and pricking sensations in lower extremities since first delivery in 1918. These symptoms are worse in cold or rainy weather. Four days before admission of baby to hospital she began to have numbness of the lips, especially the upper lip.

First child died of an unknown cause. Second died at 8 months with convulsions, cyanosis and staring eyes (Infantile beriberi), the condition lasting for 23 hours.

At present shows numbness of lips and lower extremities and diminished knee jerks.

Baby normally delivered at term. Has been entirely breast fed. Usually constipated. Has vomited at times. Two days before admission baby appeared feverish and was given a purgative. The next morning at 5:00 a. m. it began continuous convulsions, with staring of eyes and cyanosis of whole body. Admitted to hospital at 9:00 a. m. with lips cyanosed, no fever, no convulsions, urinated freely. Abdomen and extremities negative. X-ray showed heart enlarged, especially to left. Given tikitiki extract and made uneventful recovery.

A. T. 102554.

Filipino, 22 years old, mechanic. Lived in Manila last four years. Diet has been chiefly white (Saigon) rice, fish and green leafy vegetables with occasionally meat, coffee or fruit.

Mother and father living and well. Father had beriberi for three months in 1918.

In 1917 he had a "fever" for two months, followed by numbness of the extremities and paralysis of the legs rendering him unable to walk for a month. The numbness starting at this time has never entirely disappeared since. In 1919 he had "fever" for 18 days during which the numbness increased and there was marked tenderness and pain in the leg muscles which disabled him for three months. This pain and tenderness improved but the numbness had remained. In 1918 he expectorated blood and has done so since occasionally, especially after lifting heavy weights.

The present illness began one month before admission (October 21, 1922) as a "fever" lasting two weeks. Towards the end of this he felt marked numbness of both upper and lower extremities and his voice became aphonic. Extreme weakness of legs then developed and the numbness extended up to the hips and abdomen. After this his vision became "foggy." On a diet composed largely of mongo beans he improved but the numbness has persisted.

*Present condition:* Pupillary reflexes normal. Teeth and tonsils in good condition. Lungs give crackling rales over both apices, impaired resonance over left suprascapular area, diminished breath sounds over both suprascapular areas. X-ray shows heart enlarged to right, border



1 cm. to right of sternal margin. No murmurs heard. Knee jerks are sometimes obtained. There are areas of hyperesthesia paresthesia and anesthesia over both lower and upper extremities.

S. S. 104183.

Filipino, 15 years old, student. Admitted December 11, 1922, complaining of numbness of all extremities, sense of oppression in chest and fatigue on slight exertion. Diet chiefly white rice with some fish, bread and vegetables.

Had yaws five years ago, typhoid fever three years ago, and one year ago a spell of dimness or "foginess" of vision lasting one month.

The present condition began six months before admission as numbness of the extremities. Soon after there was felt a sense of oppression in the chest and of heaviness in the epigastrium. The feet and legs felt heavy. All these symptoms had improved until three days before admission when he had a fever of one night duration after which there was a return of all symptoms to an increased degree and pronounced fatigue without due exertion.

At present afebrile. Heart border 1 cm. to right of right sternal border. Apex beat in 4th and 5th interspace just inside mid-clavicular line. No murmurs heard. X-ray confirms enlargement of heart. Calf muscles and hypothenar regions tender. Walks without difficulty but cannot rise from squatting position. Knee jerks, Achilles, triceps and abdominal reflexes all absent. Numbness of hands to wrist and of dorsum of feet.

A. B. 103537.

Filipino, 18 years old, student. Admitted to hospital November 21, 1922, complaining of numbness of legs and fingers and oedema of whole body. Diet has consisted of white rice, vegetables, fish and a little meat.

Had malaria one year ago.

Present illness began eleven days before admission as numbness and swelling of the whole body. The swelling was most noticeable on the face, hands and feet, the numbness chiefly in fingers and lower extremities. Later there developed a sense of heaviness and thickness in the epigastrium and a feeling of constriction of the chest and of palpitation. Two days before admission the voice became hoarse.

On November 22, 1922 the face was swollen, lips pale. Right border of the heart 2 cm. to right of right sternal border, left border 2 cm. outside mid-clavicular line. First pulmonic sound prolonged almost to a murmur, second sound accentuated and at times reduplicated. X-ray confirms enlargement. Slight oedema over tibia and dorsum of foot. Slight tenderness of calf muscles. Diminished sensibility to pain and touch over both legs. Abdominal reflexes absent, knee jerks increased, Achilles reflexes present. Electrocardiogram showed left ventricular preponderance. Urine negative.

November 27. White blood count 8,600. Red blood count 4,440,000, 9% eosinophiles. Feces positive for ascaris and anchylostoma.

December 4. Thymol given. 26 adult anchylostomata recovered.

December 8. Red blood count 4,320,000. Hemoglobin 80%.

Eosinophiles 2%. Oedema of face, feet and legs less. Disturbances of sensation still present but improved.

L. L. 101895.

Filipino, 17 years old, student. Admitted to hospital October 1, 1922, complaining of numbness, sensation of heaviness and thickness of skin of both legs and hoarseness. Had dysentery six years ago.

Three weeks before admission woke up early with a feeling of stiffness, numbness, or heaviness and thickness of skin of both legs from ankles to knees. Walking was difficult because of weakness of legs. These symptoms increased up to the time of admission. Later he noticed pain on pressure of calf muscles and a gradual loss of ability to flex foot dorsally. Still later the disturbance of sensation appeared in the hands and the hoarseness developed.

On November 18 there was marked nystagmus of both eyes, the right pupil was dilated and the reflexes were absent. Heart enlarged to right under X-ray. Plantar, Babinski, Oppenheim, Gordon, Chaddock and knee reflexes absent. Also Achilles, patellar and ankle clonus absent. Numbness over lower leg to knee and on wrists. Areas of hyperesthesia and paresthesia over lower legs. Moderate foot drop, no wrist drop. Urine negative. Red blood count 3,000,000. White blood count 5,400. Blood negative for malaria.

January 13, 1923. Much improved. Able to walk though weak. Can rise from squatting position though with considerable difficulty. Knee jerks absent. Slight tenderness and numbness of legs. Heart somewhat smaller by X-ray.

F. C. 103618.

Filipina, 34 years old, housewife. XIII para. Admitted to hospital in labor.

Father died of beriberi, mother died in labor with 5 months fetus. 11 brothers and sisters of whom 8 are dead.

Had measles and small pox as a child. For the last 17 years has had numbness of the lower extremities which has disappeared on treatment by diet and then returned when treatment was stopped. With each pregnancy at the 8th month the numbness has spread to upper extremities, face and scalp; lasted here until the 3rd month after delivery and then receded to the lower extremities. With all of her pregnancies she has had oedema of the lower extremities, spreading over the body from the 7th or 8th month. Menstruation began when 14 years old. Married at 15. Has had 11 children, 1 miscarriage at 5 months and the present baby. Of these children, 1 died at 5 months with vomiting, 1 at 1 month, 7 days with beriberi, 2 died of small pox, 2 were stillborn, 2 died of unknown causes. In the present pregnancy the numbness of lower extremities spread up to the face and scalp and upper extremities at the 8th month. Oedema of the whole body developed at the 9th month. Labor was normal, lasting 9½ hours.

When taken for the metabolism test a week after her delivery the numbness was present over all the extremities and on the face, especially about the lips. The heart was enlarged to both percussion and X-ray. The



2nd aortic sound was accentuated. There were no murmurs. The knee jerks were increased.

L. P. 102026.

Filipino, 22 years old, laborer. Admitted to hospital complaining of numbness and weakness of lower extremities, precordial pain, hoarseness and fatigue on slight exertion.

Had usual childhood diseases, dysentery when 5 years old. Had influenza in epidemic of 1918. While convalescing from this he woke one night with "high fever" and felt numbness and tingling in legs. The next morning he noticed a slight edema of the legs. This numbness and edema increased until patient could not leave the bed for 2 months. He then improved but has had a long series of exacerbations and remissions, the symptoms lessening when he was on a diet composed largely of mongo beans and increasing again when this diet was stopped.

At the time of the metabolism test his condition was as follows: Thyroid slightly enlarged. Right border of heart 2 cm. to right of sternal border, left border just outside mid-clavicular line, X-ray showed enlargement, especially to right. 1st mitral sound weakened. Soft systolic murmur over pulmonary area, 2nd pulmonic sound accentuated. Liver edge palpable just below costal margin. No oedema of extremities. Knee jerks and Achilles reflex absent. Slight bilateral foot drop. Areas of paraesthesia and anaesthesia over both legs to knee and forearms to elbow. Urine negative. White blood count 9,400, red blood count 3,760,000, hemoglobin 80%, negative for malaria. Feces positive for ascaris and anchylostoma.

## GROUP II. NORMAL CONTROLS.

M. R.

Filipino, 22 years old, student. Admitted to hospital January 1, 1923, for relief of chronic appendicitis. Has suffered from irregular pain in right lower quadrant of abdomen for last nine months.

Physical examination negative. Reflexes normal. No tenderness or numbness of extremities.

E. M.

Filipino, 30 years old, laborer. Admitted to hospital December 26, 1922, for relief of swelling on right side of face.

Swelling began two years ago as small nodule below and anterior to lobule of left ear. Has slowly increased in size until at present it is a swelling some 8 cm. in diameter, below left ear, extending in front of and behind the ear. Swelling firm, non-fluctuating, not attached to bone or to the skin, not painful. Otherwise physical examination negative. Reflexes normal, no numbness or tenderness of muscles.

I. P.

Filipino, 22 years old, student. Admitted to hospital January 3, 1923, for relief of swelling of right sub-maxillary region.

Swelling began as small nodule in 1911 and gradually increased in size. No pain or tenderness present at any time. January 6, 1923, under local anaesthetic, two enlarged glands were found, matted together, completely



encapsulated, firm and movable. These were dissected out and removed entire. The pathologist reported "mixed tumor, sub-maxillary salivary gland."

Physical examination negative. Reflexes normal, no numbness or tenderness.

F. P.

Filipino, 17 years old, houseboy. Admitted to hospital November 29, 1922, complaining of pain in left leg of two months duration.

On admission the anterior surface of the leg was swollen and tender, the edge of the tibia presenting nodules tender to the touch. The X-ray showed no bone involvement. There was no fluctuation. The Wassermann was negative. Diagnosis of sub-acute cellulitis and periosteitis was made and the affected part treated with hot compresses. Under this treatment the pain and tenderness subsided. When run as a metabolism control, December 12, 1922, the leg was normal in appearance and in feeling and the patient was afebrile. Physical examination negative. Reflexes normal. No numbness or tenderness.

M. B.

Filipino, 14 years old, houseboy. Admitted to hospital December 9, 1922, complaining of painful swelling of scalp in left parietal region.

Bumped head against stone wall December 3, 1922. Immediate pain and swelling. On admission presented a painful swelling of scalp over left parietal region. X-ray showed no sign of fracture. Hematoma aspirated December 11, 1922, with relief of all symptoms.

A slight, well nourished boy. Physical examination negative except for area of tenderness still remaining in left parietal region. Reflexes normal. No numbness or tenderness of muscles.

D. R.

Filipino, 19 years old, student. Admitted to hospital November 22, 1922, for operation for left inguinal hernia of 2 years' duration. Herniotomy performed November 22, 1922, under local anaesthetic.

Knee jerks, triceps and biceps reflexes very active. No numbness, weakness or tenderness. Physical examination otherwise negative.

T. V.

Filipino, 25 years old, student. Admitted to hospital December 16, 1922, for relief from external hemorrhoids of several months' duration.

Knee jerks diminished. Physical examination otherwise negative. No numbness or tenderness of muscles, no foot drop.

D. C.

Filipino, 30 years old, driver. Admitted to hospital December 15, 1922, with stab wound, non-penetrating, in 7th left lateral interspace. Wound dressed. Recovery uneventful.

A well nourished man much more heavily muscled than the average Filipino. Physical examination negative except for active knee jerks and biceps reflex. No foot drop, no tenderness or numbness.

The results obtained in the metabolism determinations for the two groups are given in Table III and Table IV. For purposes of comparison

TABLE III.  
Metabolism Data in Beriberi Patients.

Name	Date	Area in sq. Meters	B. M. R. plus minus	B. M. R. corrected to Non-protein R. Q.	R. Q.	Non-protein R. Q.	Urinary nitrogen gm. per hr.	Oxygen absorbed, liters per hr.	Carbon dioxide produced, liters per hr.	Total Calories per hr.	Calories from protein	Calories from fat.	% of Total Calories	Calories from Carbohydrate	% of Total Calories
R. I. 102418	2 Dec. 1922	1.62	1.4% plus	1.6% minus	0.821	.0848	0.340	12.130	10.196	58.20	9.01	25.09	15.5	24.10	43.1
C. P. 103692	27 Nov. 1922	1.43	7.3% minus	8.1% minus	0.810	0.811	0.274	10.210	8.271	48.62	7.26	26.72	14.9	14.64	54.9
A. T. 102554	21 Nov. 1922	1.42	10.8% minus	12.2% minus	0.813	0.815	0.418	10.837	8.442	49.26	11.08	24.66	22.5	13.52	50.1
S. S. 104183	14 Dec. 1922	1.45	15.0% minus	15.9% minus	0.800	0.799	0.440	11.846	9.476	56.11	10.60	30.95	18.9	14.56	55.1
.....	18 Jan. 1923	1.46	0.3% plus	2.4% minus	0.930	0.990	0.665	12.110	11.265	58.41	17.63	1.38	30.2	39.39	2.4
A. B. 103537	22 Nov. 1922	1.47	3.4% minus	4.4% minus	0.834	0.840	0.311	12.007	10.024	57.56	8.24	26.83	14.3	22.49	46.6
.....	9 Dec. 1922	1.37	1.7% minus	3.7% minus	0.829	0.839	0.564	11.415	9.467	54.14	14.95	21.32	28.3	18.87	39.4
L. L. 101895	18 Nov. 1922	1.33	5.6% minus	6.5% minus	0.775	0.770	0.297	11.315	8.722	53.42	7.87	35.62	14.7	9.93	66.7
.....	13 Jan. 1923	1.39	15.3% minus	16.2% minus	0.836	0.841	0.263	10.436	8.720	50.01	6.97	23.39	13.9	19.65	46.8
F. C.	5 Dec. 1922	1.43	0.8% minus	1.9% minus	0.809	0.810	0.286	10.759	8.704	51.22	7.58	28.19	14.8	15.45	55.0
L. P.	24 Nov. 1922	1.58	10.4% minus	12.2% minus	0.793	0.789	0.495	11.646	8.721	54.88	13.12	29.82	23.9	11.98	54.3
Average	.....	1.45	6.3% minus	7.7% minus	0.823	0.832	0.388	11.337	9.273	48.84	10.39	24.91	19.2	18.59	46.7

TABLE IV.  
Metabolism Data in Normal Controls.\*

Name	Date	Area in sq. Meters	B. M. R. minus 3.5% plus 17.0% minus 13.7% minus 1.0% minus 5.3%	B. M. R. corrected to R. Q. minus 2.3% minus 8.2% minus 7.5% minus 4.6% minus 15.4% minus 2.8% minus 8.3%	R. Q.	Non-protein R. Q.	Urine nitrogen gm. per hr.	Oxygen absorbed, liters per hr.	Carbon dioxide produced, liters per hr.	Total Calories per hr.	Calories from protein	% of Total Calories	Calories from fat.	% of Total Calories	Calories from Carbohydrate	% of Total Calories
M. R.	4 Jan. 1922	1.73			0.881	0.900	0.472	13.463	11.855	62.67	12.51	20.0	17.03	27.2	33.13	53.4
E. M.	28 Dec. 1922	1.50			0.870	0.881	0.277	11.943	10.396	57.85	7.34	12.69	20.61	35.62	29.90	51.69
I. P.	15 Jan. 1923	1.69			0.798	0.796	0.556	13.010	10.380	61.33	14.74	24.0	33.27	54.2	13.32	21.7
F. P.	12 Dec. 1922	1.36			0.832	0.841	0.476	11.368	9.458	54.12	12.62	23.3	22.58	41.7	18.92	35.0
M. B.	15 Dec. 1922	1.20			0.854	0.977	0.465	11.830	10.107	52.89	12.33	23.1	2.76	5.2	37.80	71.5
D. R.	16 Dec. 1922	1.42			0.834	0.883	0.231	10.207	8.911	49.48	6.12	12.4	17.69	35.7	25.67	51.9
T. V.	19 Dec. 1922	1.45			0.852	0.872	0.550	10.178	8.670	48.46	14.58	30.1	14.97	30.9	18.91	39.0
D. C.	26 Dec. 1922	1.57			0.911	0.932	0.470	12.423	11.226	60.32	12.46	20.7	11.39	18.9	36.47	60.5
Average	.....	1.49			0.854	0.886	0.437	11.803	10.125	56.51	11.59	20.8	17.66	31.2	26.77	48.1



the averages obtained in twenty cases of Americans, made in Washington, D. C., in 1921, are given in Table V. These were cases of neurasthenia and various minor surgical traumata in American soldiers and two cases of non-toxic goiter of adolescence in applicants for the Army School of Nursing. There is also given for this purpose in Table VI a copy of part of a table of basal metabolic rates found in a similar group by Boothby and Sandiford.<sup>11</sup> Because of the long delay in mails between the States and Manila, the liberty has been taken of printing this extract without authorization of the authors. Full acknowledgement is made to them as the source of the table.

TABLE V.

*Metabolism Data in Normal Americans.*

Area in sq. meters .....	1.71
B. M. R. ....	-1.7% minus
B. M. R. corrected to Non-protein R. Q.....	2.4% minus
R. Q. ....	0.795
Non-protein R. Q. ....	0.794
Urinary nitrogen .....	
Oxygen absorbed, liters per hr. ....	13.500
Carbon dioxide produced, liters per hr. ....	10.495
Per cent. of total Calories from protein combustion.....	15.20
Per cent. of total Calories from fat combustion.....	59.81
Per cent. of total Calories from carbohydrate combustion.....	22.66

TABLE VI.

*\*Basal metabolic rates found in 455 persons by Boothby and Sandiford*

Diagnosis	No. of Cases	B. M. R.
Chronic nervous exhaustion .....	249	1.2% plus
Migraine .....	29	0.7% minus
Obesity .....	73	0.7% minus
Normal .....	102	0.6% plus

Examination of these results gives no constant differences between the two groups which can be taken as significant of beriberi. The greatest difference between normal Filipinos and beriberi patients is apparently in the per cent. of heat derived from fat and from carbohydrate combustion respectively. However, the still lower per cent. of heat from carbohydrate in the group of Americans is evidence against the lower figure in beriberi signifying a decrease in the ability to burn carbohydrate. It is taken to be a chance variation between small groups. The

\* An extract from Table V, page 799 of "Summary of the Basal Metabolism Data on 8614 Subjects with Especial Reference to the Normal Standards for the Estimation of the Basal Metabolic Rate" by Walter M. Boothby and Irene Sandiford. J. Biol. Chem., Vol. 54, No. 4, December, 1922.

respiratory quotients found are consistent with the findings of Anderson and Kulp<sup>12</sup> that vitamine starvation produces no change in the respiratory quotient and with the similar results of Jansen and Mangkoewinoto.<sup>13</sup> It is, of course, possible that a person suffering from the clinical syndrome known as beriberi need not be in the stage of actual vitamine deficiency at that particular time. All the beriberi patients, while in hospital, were constantly on a diet calculated to meet all vitamine requirements. The clinical picture seen may be the residual effect of previous vitamine deficiencies manifested by various nerve degenerations, slow of recovery, while, at the same time, the metabolism may have been restored to normal by the diet given in treatment. Cases were examined in various stages of the disease and of treatment but no change of respiratory quotient referable to amount of vitamine available to the body was found. The effect of tikitiki extract\* on the respiratory quotient of experimental animals kept on a diet of polished rice is to be investigated by this Board.

As with the basal metabolic rate, so also has the chemistry of the blood, as far as investigated in this work, failed to show changes that can be held characteristic of beriberi. The result of blood chemistry determinations in beriberi cases and in normal Filipinos are given in Tables VII. and VIII.

It is thought, therefore, that the data found for both normal and beriberi patients may be taken as equally representative of the normal Filipino.

Read and Wang<sup>5</sup> conclude that the low nitrogen output found by all investigators of tropical metabolism is due to the low protein intake and not to high environmental temperature and humidity. Their work was done in Peking "in an exceedingly dry climate and mostly during the winter when snow was falling"; conditions in marked contrast to those obtaining in India and the Philippines. The figures obtained by them and others—McCay,<sup>1</sup> Aron,<sup>2</sup> Campbell,<sup>3</sup> Concepcion<sup>4</sup>—as to the nitrogenous end-products of metabolism are in substantial accord with the figures here presented for the gaseous end-product of the same process and with certain of the nitrogenous products carried in the blood

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\* Tikitiki extract is an extract of TIKITIKI or rice bran, removed in polishing the rice for market. The bran is extracted with 25 per cent. alcohol, this extract evaporated to a syrup in vacuum, clarified by standing and centrifuging, precipitated by 95 per cent. alcohol and again evaporated to a syrup after filtration. This is then bottled, pasteurized for three successive days and distributed. In the Philippines this is used largely in the treatment of the infantile form of beriberi.

TABLE VII.  
Blood Chemistry in Beriberi Patients.  
Figures represent mg. per 100 cc. whole blood.

Name	Date	Sugar	Non Protein Nitrogen	Uric Acid	Urea Nitrogen	Urea	Creatinine
R. I. 102418	22 Nov. 22	92	35.3	3.7	13.0	28.1	1.4
----- C. P. 103694	2 Dec. 22	102	34.2	4.3	19.3	41.8	1.1
* * M. S. A. T. 102553	27 Nov. 22	102	23.8	3.3	9.1	19.9	1.3
* S. S. 104183	25 Nov. 22	89	37.8	5.8	13.0	28.0	2.2
----- A. B. 103537	9 Nov. 22	105	29.2	3.4	13.6	29.5	1.7
----- L. L. 101895	21 Nov. 22	148	34.2	3.4	10.5	22.7	1.5
----- F. C. 103618	14 Dec. 22	89	30.8	4.6	10.5	22.7	1.3
----- L. P. 102026	18 Jan. 23	102	27.2	3.4	10.0	21.6	1.0
----- Averages of 29 analyses on above and other beriberi cases.	22 Nov. 22	100	33.3	4.3	12.2	26.5	1.5
-----	9 Dec. 22	102	35.3	4.4	15.0	32.2	1.4
-----	4 Nov. 22	108	30.0	3.2	12.9	27.6	1.3
-----	18 Nov. 22	105	34.2	3.0	18.3	39.5	1.6
-----	5 Dec. 22	100	21.6	4.0	10.9	23.6	1.4
-----	4 Nov. 22	117	32.4	3.7	13.6	29.5	1.5
-----	24 Nov. 22	100	33.3	3.9	13.0	28.1	1.7
-----	-----	102	31.5	3.7	12.9	28.1	1.4
-----	-----	107	31.3	3.5	12.7	27.4	1.4

\* \* Infant of C. P. above. Taken day after recovery from acute attack of infantile beriberi. Figures not included in averages.

\* Sugar value probably about 40 per cent. too high due to deteriorated standard from mold contamination. Factor of correction found by checking this old standard against standard freshly made. Figure not used in computing average.

TABLE VIII.  
Blood Chemistry in Normal Controls.  
Figures represent mg. per 100 cc. whole blood.

Name	Date	Sugar	Non Protein Nitrogen	Uric Acid	Urea Nitrogen	Urea	Creatinine
M. R.	4 Jan. 23	93	33.3	4.0	9.8	21.2	1.5
E. M.	28 Dec. 22	98	27.2	4.4	8.4	18.1	1.6
I. P.	15 Jan. 23	100	27.2	3.9	9.7	22.0	1.4
F. P.	12 Dec. 22	93	30.0	4.4	12.0	26.0	1.4
M. B.	15 Dec. 22	114	30.0	4.8	9.4	20.2	1.3
D. R.	16 Dec. 22	111	-----	-----	-----	-----	-----
T. V.	19 Dec. 22	100	32.4	3.2	13.0	28.1	1.5
D. C.	26 Dec. 22	160	25.0	4.0	11.3	24.5	1.4
Average	-----	109	29.3	4.1	10.5	22.8	1.4



before excretion. In considering the values for uric acid it is to be remembered that the new method of Benedict gives higher readings than that of Folin upon which most of the figures in the literature are based. All figures point to a level of metabolism lower than that found in the States. The low values found for urea and creatinine in the blood and the low nitrogen content of the diet make one feel that this lowered level is due to low protein intake. However, from the urinary nitrogen and the partition of metabolism by means of the non-protein respiratory quotient between protein, fat and carbohydrate combustion it is seen that the Filipino group obtains fully as high a percentage of the total heat from combustion of protein as does the American group.

The blood sugar values fall well within the normal range for Americans. This is in contrast to the very high carbohydrate content of the diet and is in accord with the belief that diabetes is not a common disease among Filipinos.<sup>†</sup> The respiratory quotients found also show no impairment in the ability to burn carbohydrate. The average blood sugar level found is much lower than that found in Filipinos by Concepcion.<sup>14</sup> Using the Meyers-Bailey method he found an average of 123 mg. per 100 c. c. in a series of thirty normal cases.

The level of the blood creatinine is also low, but here again the difference between the values found and those accepted as normal for Americans (1.5—1.7 mg. per 100 c. c.) is too small to be of aid in definitely answering the question of the cause of the low metabolism. However, it is consistent with the theory of a low protein intake. For the present it can only be said that the basal metabolism in normal Filipinos appears to be some six per cent. lower than in normal Americans. Whether this is due to a meager diet, especially low in protein, to a higher environmental temperature and humidity, or to an endogenous metabolism essentially low cannot be stated. Work on another group of Filipinos is planned with this question in mind.

### *Summary*

1. Between two groups of Filipinos, one with the confirmed diagnosis of beriberi, the other surgical convalescents, no essential differences were found in the basal metabolism rate, respiratory quotient, or blood chemistry.

<sup>†</sup> For the years 1914, 1916, 1918, 1919, 1921, Manila with a population of about 260,000 had 40 deaths reported from diabetes among Filipinos.

2. Considering both groups together, the basal metabolic rate was found to average six per cent. minus.

3. In both groups the non-protein nitrogen, urea nitrogen and creatinine were on the low end of the range considered normal for Americans. Uric acid appears to be as high, if not higher, than in Americans. The values for blood sugar come within the normal range.

4. Evidence is presented both for and against the low metabolic rate being due to low protein intake.

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## TIME RELATION OF THE FALL OF BLOOD SUGAR WITH INSULIN.

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In an attempt to discover the direct effect, if any, of insulin on glucose, 0.1 cc. of iletin and 2 cc. of glucose solution were put in a test tube, and the mixture observed at various intervals to see whether or not there was any digestion or disappearance of the glucose. Table I shows that no change took place even after the mixture had been allowed to stand at room temperature for 150 minutes.

As a second test, I added to a like mixture of iletin and glucose,  $\frac{1}{2}$  cc. of blood, the blood sugar content of which was 89 mg. per 100 cc. (taken from a normal individual, viz., the writer) and allowed the mixture to stand for 150 minutes. As shown by Table II no change was observed excepting a slight fluctuation which can easily be attributed to a technical error in reading such high sugar values.

A third test was the same as the preceding excepting that 0.2 cc. of insulin were used in place of 0.1 cc. with the same result. See Table III.

These findings tend to indicate that insulin produces no reaction upon either blood or glucose, in vitro. In spite of the fact that the iletin concentration in these experiments was much higher than it is in the body, in  $2\frac{1}{2}$  hours it had no effect. These results agree with the findings of the Toronto investigators.<sup>1</sup>

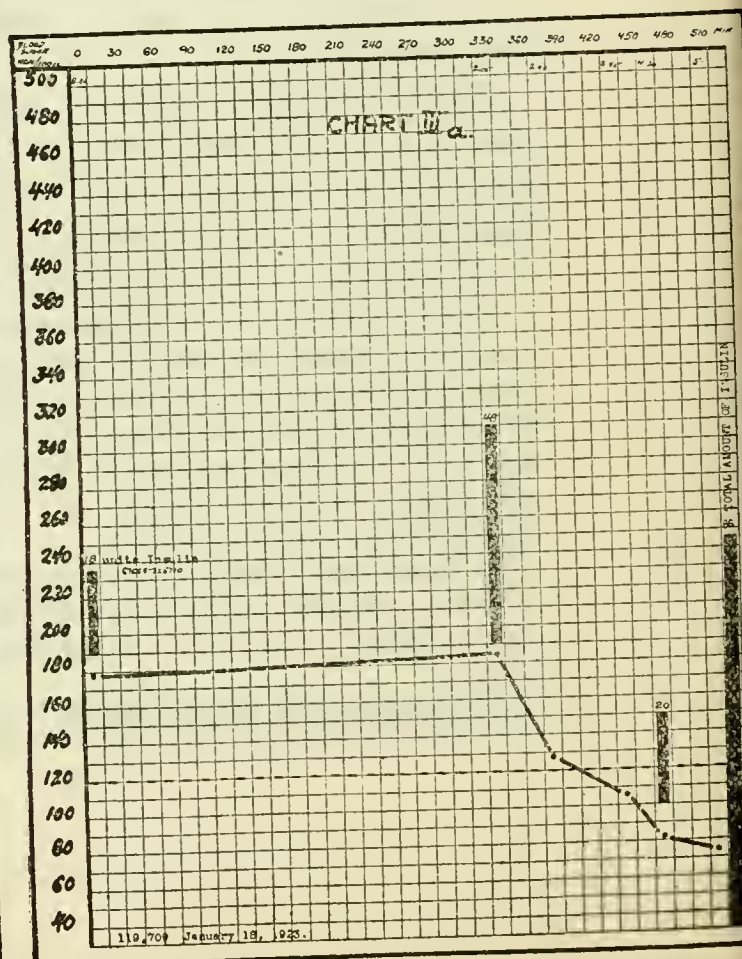
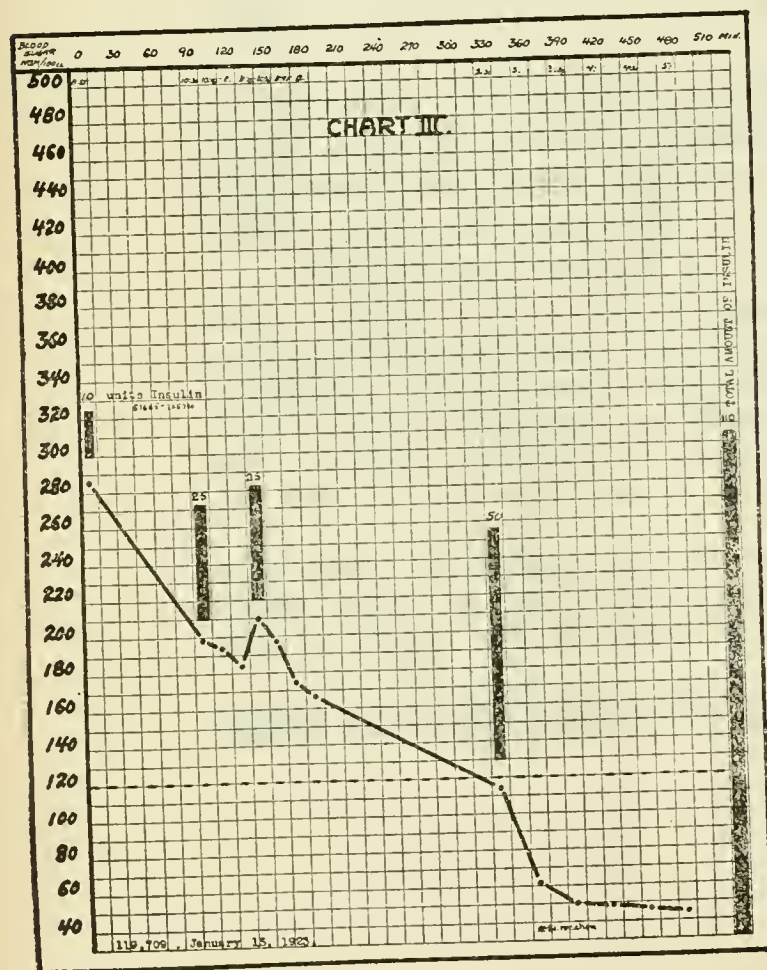
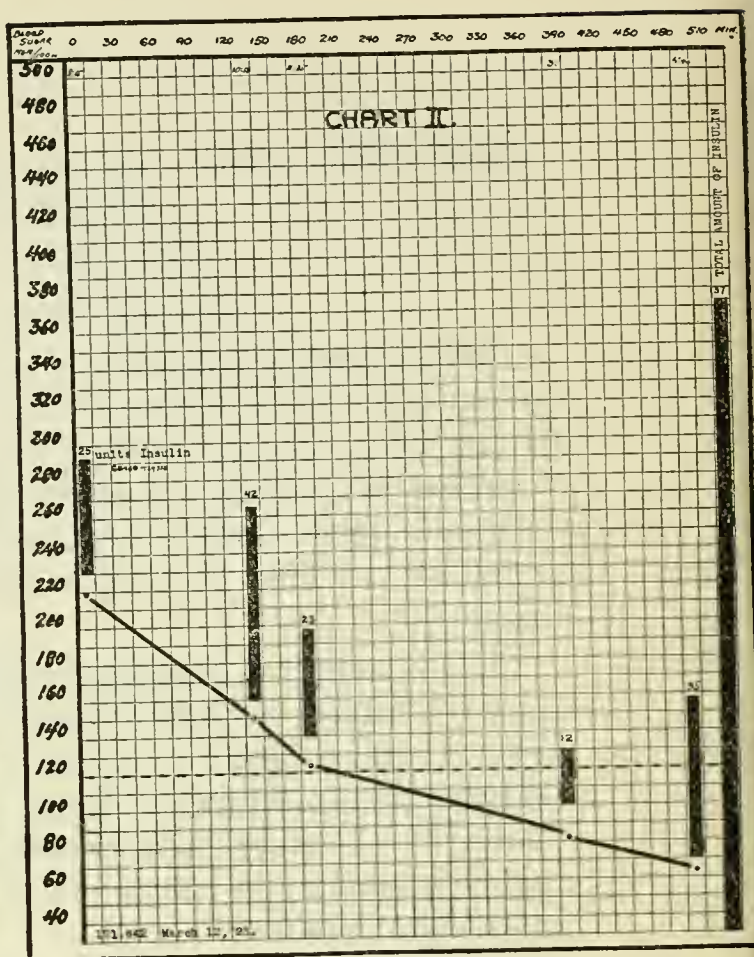
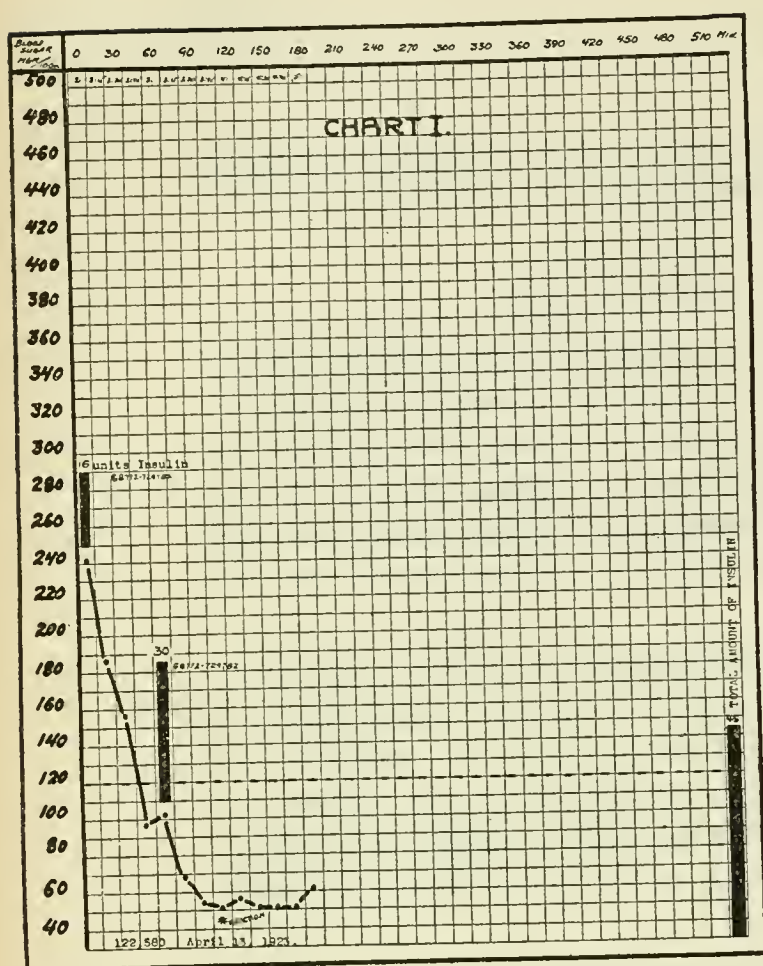
These changes are shown graphically, not only in their relation to the dosage of insulin but also to the time within which they occurred. Each chart represents the reaction in one patient during one day, although as will be noted, in some cases the insulin was repeated on several days. All the injections of insulin were given intravenously. When it was practicable to do so, blood was taken every fifteen minutes after the administration of the insulin.

In some cases the periods between the injections of the insulin were longer, as will be noted on examination of the charts. As all are plotted on a uniform scale, they can readily be compared. The number of units

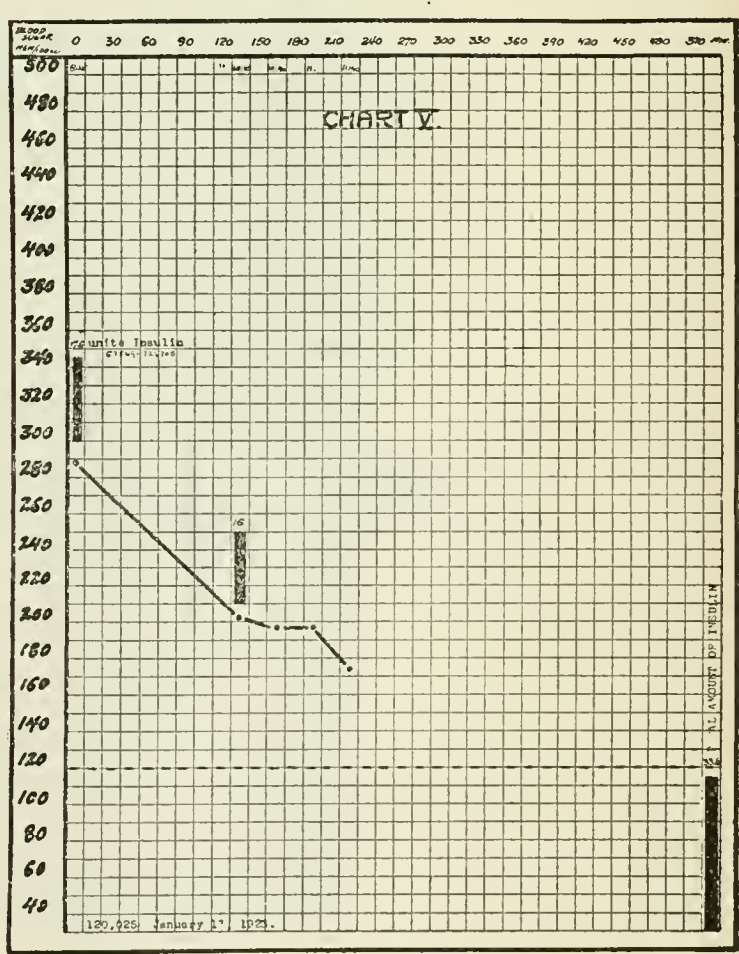
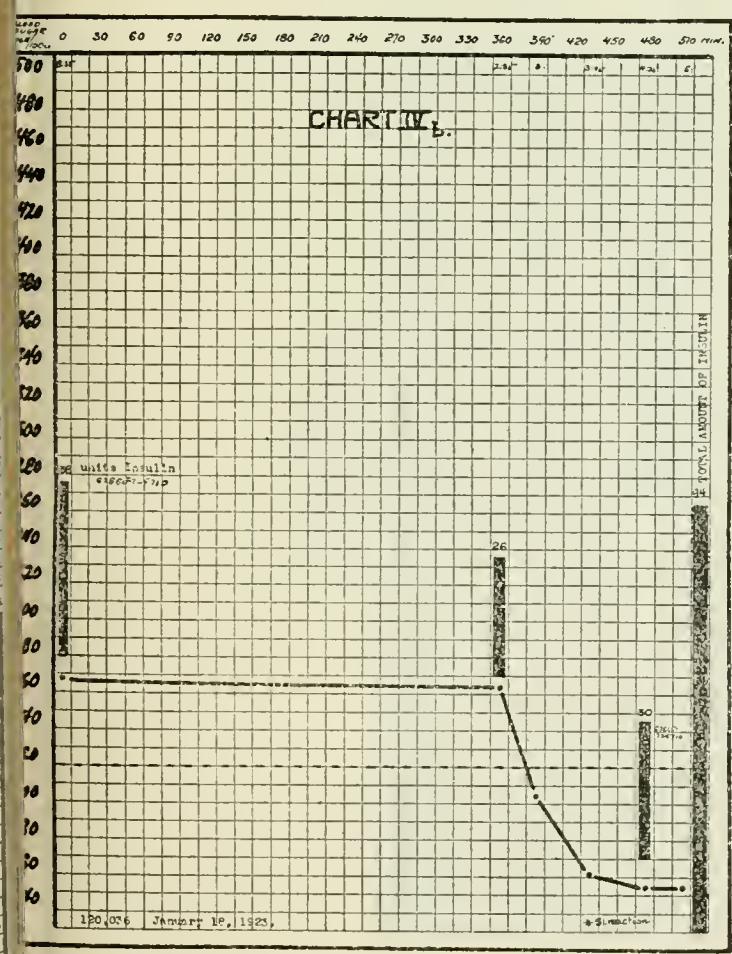
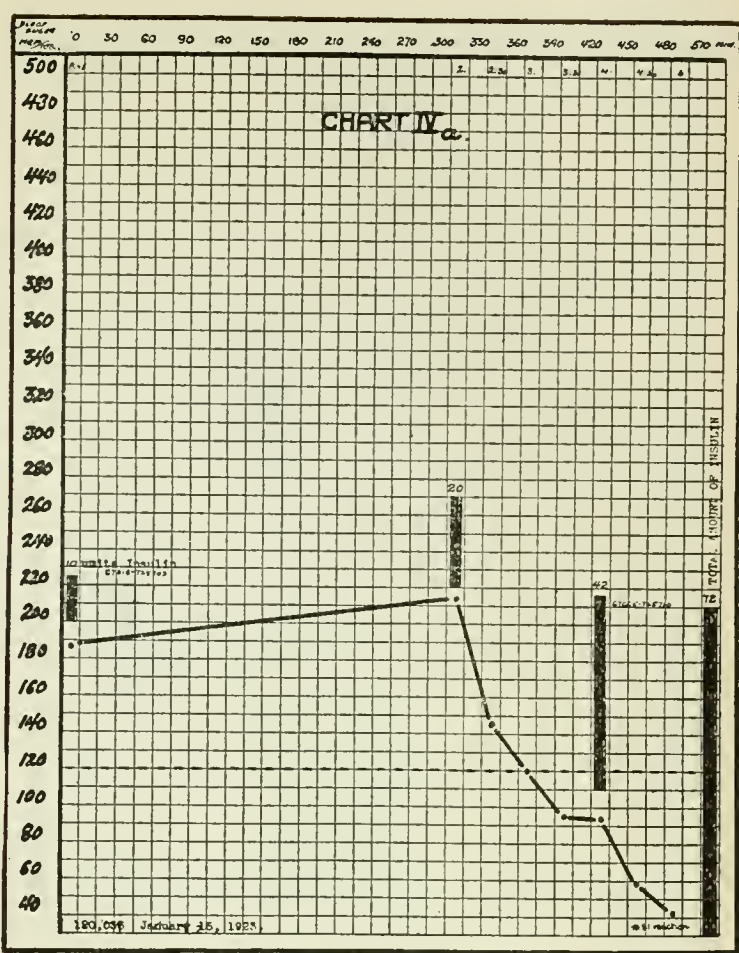
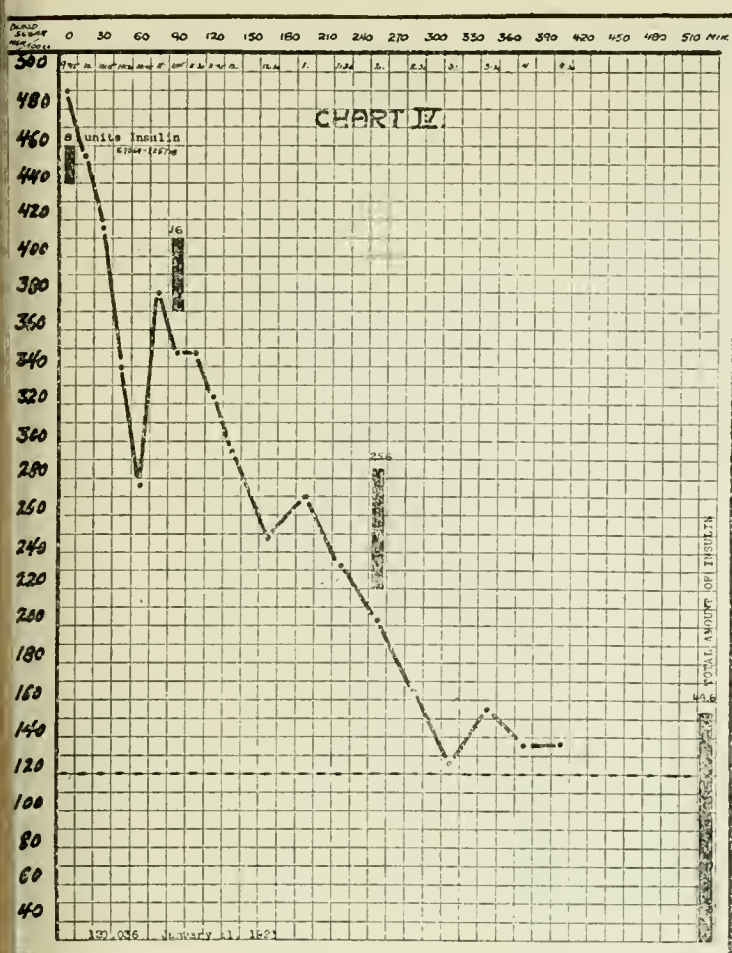
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\* The insulin used in this investigation has been that prepared by Eli Lilly and Company under the name of Iletin. The dosage is expressed in the units as defined by this company.

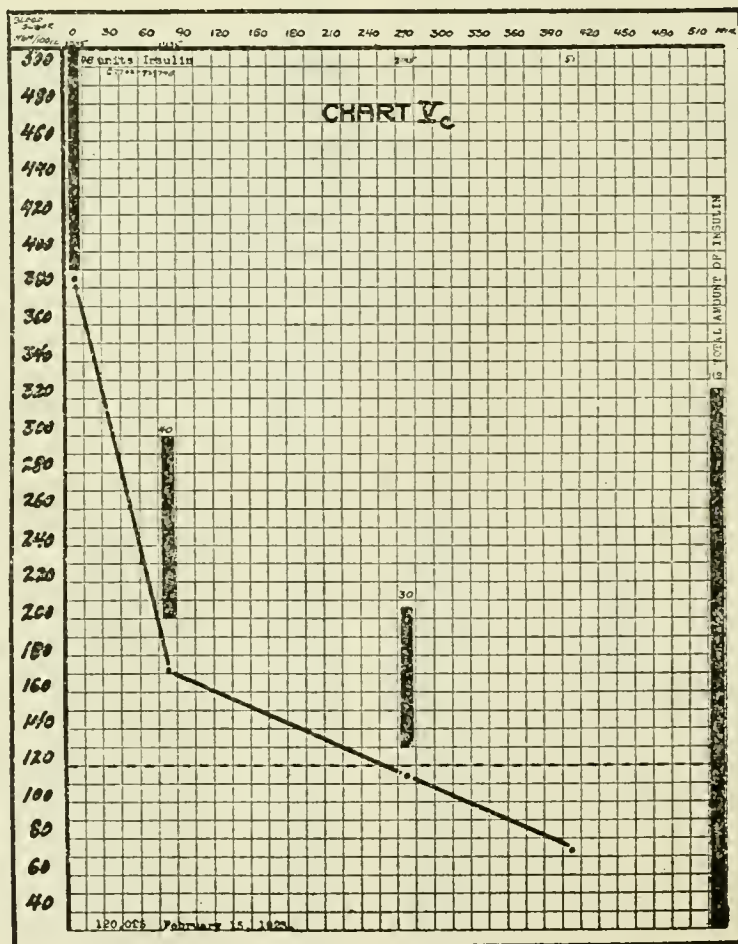
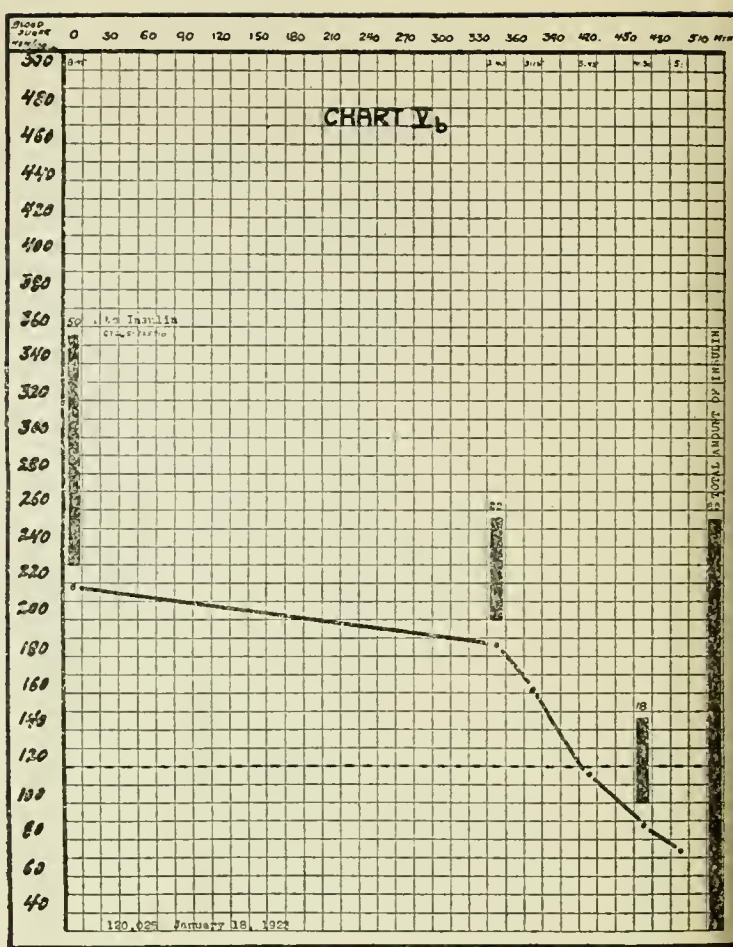
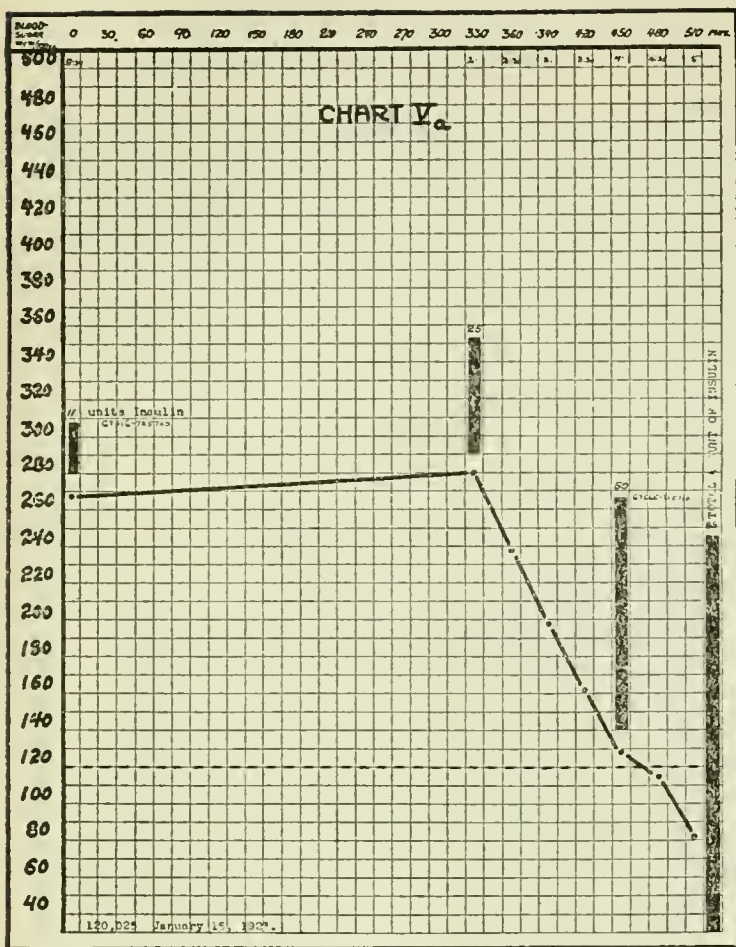
The following charts have been made in a study of the changes in the blood sugar content which follow the administration of insulin\* to diabetic patients.

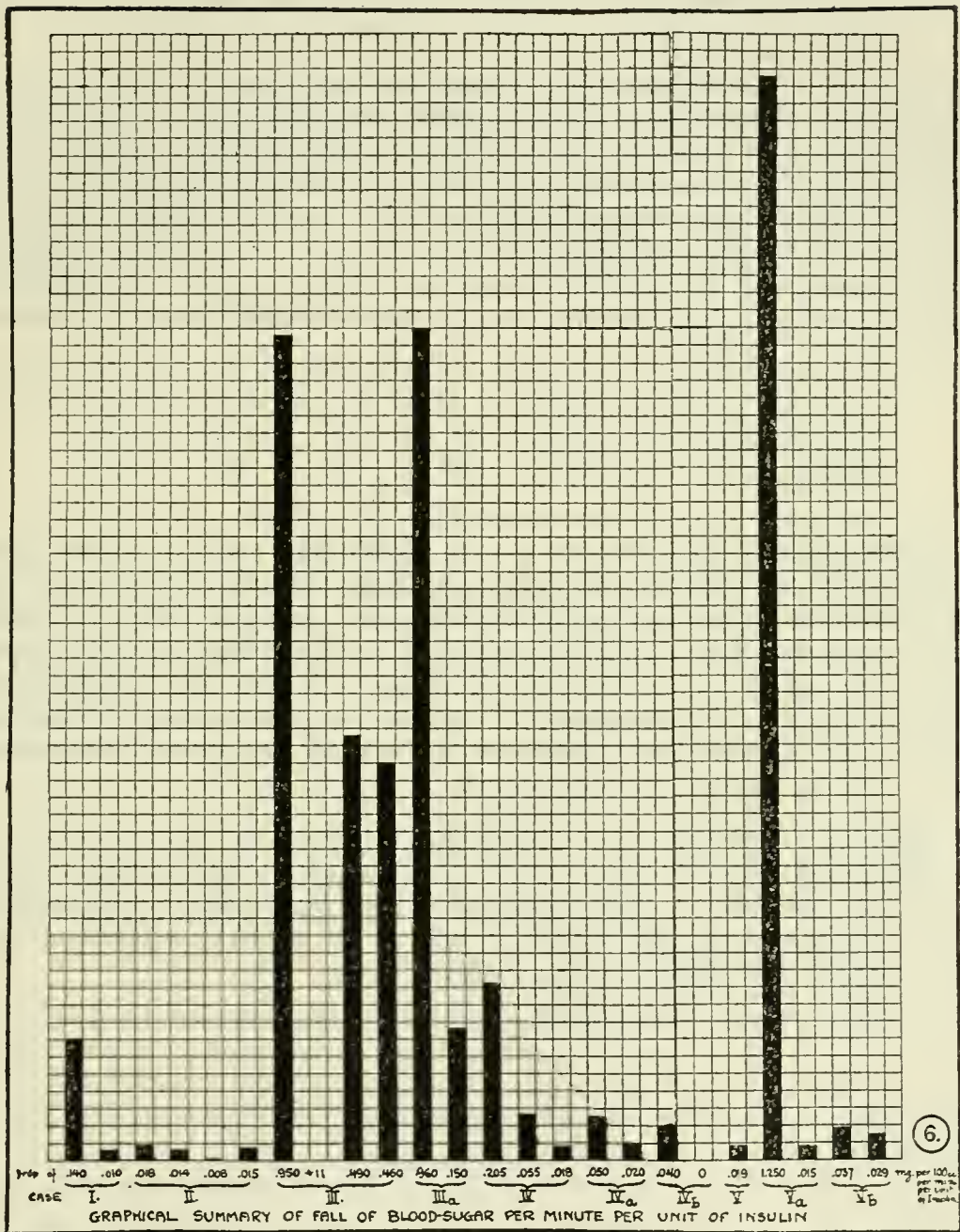














of insulin given in each dose is shown by the black columns in which each square represents four units. The total amount of insulin given on each day is shown by the black column at the right of each chart, the number of units in each case being given in figures at the top of each column. Whenever a reaction, due to hypoglycemia, followed the dose of insulin, this is denoted by an asterisk beneath the blood sugar curve.

Case No. 1. This patient, a young man 32 years of age, had had diabetes for about 10½ months, during which period he had lost 50 pounds. He had been treated for stomach trouble, the diabetes being unrecognized, and his urine had not been examined. When he came to the Clinic his blood sugar was 696 mg. per 100 cc. and the sugar content of the urine was 12 per cent. For four days he was kept on a diet consisting of carbohydrate 30 gm., protein 30 gm., fat 40 gm., a total of 600 calories. During these four days his blood sugar showed the following reductions:

Date—April.....	9	10	11	12	13
Blood Sugar.....	696	309	254	204	213

On the fourth day, I decided to use insulin and gave it to him in the dosage shown in Chart 1. As shown by the chart, the blood sugar fell, a mild reaction following the second injection when the blood sugar value was 51 mg. per 100 cc. This reaction was expressed by sweating, palpitation of the heart and a subjective feeling of prostration. Nothing was given to counteract this effect, the blood sugar remaining at this low level for almost two hours, when the patient ate his dinner, immediately after which he again felt quite normal.

Case No. 2 was that of a young man 32 years of age, with history of diabetes of 2 years' duration. He came in with a blood sugar content of 552 mg. per 100 cc. He was given a diet consisting of carbohydrate 30 gm., protein 30 gm., fat 40 gm., a total of 600 calories, and was at once given insulin, with the following result:

Date—March.....	7	8	9	10	11	12
Blood Sugar.....	552	246	234	200	.....	219
Insulin Units.....	.....	18	25	8	.....	137

The study of the glycemia on March 12 started at 8:15 A. M., and lasted until 4:40 P. M. Thus, following the first dose he had his breakfast and between the third and fourth doses lunch was taken. The total dosage on this date was large, namely 137 units, but although the blood sugar dropped as low as 63 mg. per 100 cc., there was no reaction. The last injection no doubt was followed by a further drop, but as the patient received his dinner shortly afterward this was compensated for, and a normal glycemia was restored. On the following morning the blood sugar



was 228 mg. per 100 cc., i.e., at a higher level than on the preceding morning, in spite of the large total dosage of insulin. The observation of this case would tend to indicate that a lasting reduction of the blood sugar is not related to the quantity of insulin received, but must be due to some other factor, probably the condition of the pancreas, viz., the islands of Langerhans.

Case No. 3. This patient was a young man 28 years of age, with diabetes of long standing—5 years. He was markedly emaciated, having been on a low diet for months before he came to the clinic. He came in with a blood sugar content of 319 mg. per 100 cc. On a diet of 30 gm. carbohydrate, 30 gm. protein, and 29 gm. fat, a total of 500 calories, and with daily doses of insulin, his blood sugar showed the following changes:

Date—Jan.....	3	4	5	6	7	8	9	10	11	12	13	14	15
Blood Sugar....	319	246	232	261	.....	236	212	240	265	206	246	.....	285
Insulin Units....	43.6	28.8	33.6	16	.....	50.2	17.6	22.4	46.4	16	24	.....	110

This was a very severe case of diabetes and the daily administration of insulin did not have much effect on his blood sugar, which remained at about 250 mg. per 100 cc. Yet the man felt better. After each administration of insulin, he experienced a feeling of light heartedness and of perfect content. How much of this was psychic and how much physical is hard to determine, for in some cases the extensive newspaper propaganda regarding insulin, in which it has been described as "a cure for diabetes," has raised the hopes of these patients unduly, so that it is hard properly to evaluate the psychic factor.

In this case the study of the reaction which is shown in the chart was started at 8:55 A. M., and ended at 5:00 P. M. The first injection was followed by breakfast, and the blood sugar value of 166 mg. per 100 cc. in the middle of the chart just preceded luncheon.

In this case as in the preceding, the blood sugar fell continuously and at a fairly uniform rate. When the blood sugar content was 61-50 mg. per 100 cc. there was a slight reaction manifested by profuse perspiration, palpitation of the heart and a feeling of weakness.

From this date (Jan. 15) to Jan. 18, when Chart III (a) was made, the patient was on a diet of 30 gm. carbohydrate, 30 gm. protein, and 56 gm. fat, a total of 750 calories, and continued to receive insulin. The daily blood sugar values were as follows:

Date—Jan.....	15	16	17	18
Blood Sugar.....	285	184	165	178
Insulin.....	110	24	22	86

On Jan. 18, the observations shown on Chart III (a) were made. The first blood sugar value on this date was determined at 8:30 A. M., before

breakfast; the second at 2:05 P. M., i.e., after luncheon, following which there was an uninterrupted and uniform fall of blood sugar down to 75 mg. per 100 cc. without any reaction.

Case 4. This patient was a young man of 22 years of age, in whom diabetes had recently developed following a gonorrheal infection. He came to the clinic on Jan. 11 with a blood sugar content of 490 mg. per 100 cc. He was put on a diet consisting of 30 gm. carbohydrate, 30 gm. protein, 29 gm. fat, a total of 500 calories. On this date, as shown by Chart IV, 19 blood sugar estimations were made. He had no breakfast and no luncheon; so that in this case the progress of the blood sugar changes was in no way influenced by his diet.

This was a recent case of diabetes, which as the chart shows responded promptly to small doses of insulin. Within 7 hours the blood sugar had fallen from 490 mg. per 100 cc. to the normal level, with a total dosage of 49.6 units of insulin.

Between Jan. 11 and Jan. 15, the blood sugar changes were as follows:

Date—Jan.....	11	12	13	14	15
Blood Sugar.....	490	197	194	....	187
Insulin.....	49.6	8	28.8	....	72

On Jan. 15, the observations shown on Chart IV (a) were made, the first estimation being made at 8:45 A. M., before breakfast, and the next at 2:00 P. M. When the blood sugar reached 59 mg. per 100 cc. there was a slight reaction like those described above. A total of 72 iletin units was given during this day.

Between Jan. 15 and 18, the blood sugar changes on a diet consisting of 30 gm. carbohydrate, 30 gm. protein, 56 gm. fat, a total of 750 calories, were as follows:

Date—Jan.....	15	16	17	18
Blood Sugar.....	187	185	193	168
Insulin.....	72	20	30	64

On Jan. 18 (Chart IV, b), the first blood sugar estimation was made at 8:35 A. M., before breakfast; the second, at 2.35 P. M., after lunch; the observation ending at 5:00 P. M., before dinner. This chart again shows a uniform fall of blood sugar after the second dose of iletin, down to 54 mg. per 100 cc. When the blood sugar reached 61 mg. per 100 cc., there was again a slight reaction.

Case No. 5. This patient was a young man 28 years of age, with a severe diabetes of 3 months' standing which had been preceded by no infectious disease. He was markedly emaciated and toxic; showed considerable acetone in the plasma and lipemia as well. His blood sugar on admission was 696 mg. per 100 cc. with 3.4 per cent. of sugar in the

urine. He was put on a diet of 30 gm. carbohydrate, 30 gm. protein, 28 gm. fat, a total of 500 calories. The blood sugar changes during the first three days were as follows:

Date—Jan.....	11	12	13
Blood Sugar.....	696	336	288
Insulin.....	16	17.6	33.6

The observations shown in Chart V were started at 8:25 A. M., before breakfast and ended at 11:40 A. M., before lunch; thus breakfast was the only dietary factor affecting these values. This chart shows a gradual fall of the blood sugar from 288 to 174 mg. per 100 cc., without any reaction.

Two days later on Jan. 15, the observations shown on Chart V (a) were made. The first blood sugar estimation was made at 8:34 A. M., before breakfast; the next at 2:00 P. M., after luncheon. In this chart as in Chart V, there was again the gradual and uniform fall of the blood sugar from 280 to 82 mg. per 100 cc., without any reaction or any atypical condition.

Three days later, on Jan. 18, the first estimation was made at 8.45 A. M., before breakfast; the second at 2:40 P. M., after luncheon, and the final estimation at 5:00 P. M., before dinner. Chart V (b).

The striking factor in this chart is the large initial dose of insulin, namely 50 units, which was followed by no reaction. The subsequent doses were followed by a steady fall of the blood sugar to 73 mg. per 100 cc., without any reaction. This man received 90 units of insulin in 8 hours and 25 minutes, with a resultant fall of his blood sugar content from 219 to 73 mg. per 100 cc.

On Feb. 15, the observations shown in Chart V (c) were made. The first blood sugar estimation was made at 10:15 A. M., before breakfast; luncheon was taken after the second, and the test ended at 5:00 P. M., before dinner. The blood sugar fell progressively to 73 mg. per 100 cc., without any reaction, in spite of the large total dosage of 118 insulin units.

After Jan. 31, this patient began to develop a series of acute colds—coryza—together with some bronchitis. This explains the sudden rise of blood sugar after that date. Moreover, he had left the hospital and I had my doubts about his fidelity to the prescribed diet. He subsequently ate without restraint and died on March 18.

This patient had a severe case of diabetes. He responded promptly to insulin and we were able to keep his blood sugar down to a safe level, up to the time of the onset of the acute colds. He then gave up the struggle. The result emphasizes the fact that a cold may be a very serious danger to a diabetic patient if it is not promptly looked after.

An attempt has been made to correlate the dosage of insulin with the period within which the effect first becomes manifest and the duration of those effects. Examination of the individual





charts will show at once the difficulty of establishing such a relation at present. In the following Table, I have attempted to show the effect in each instance of a single unit of insulin calculated in terms of the fall of blood sugar per minute.

FALL OF BLOOD SUGAR FOLLOWING INSULIN ESTIMATED  
PER MINUTE PER UNIT OF INSULIN

Case No.	DOSE I		DOSE II		DOSE III		DOSE IV	
	No. of units	Fall per min. per unit	No. of units	Fall per min. per unit	No. of units	Fall per min. per unit	No. of units	Fall per min. per unit
1	16	.140	30	.010	-----	-----	-----	-----
2	25	.018	42	.014	23	.008	12	.015
3	10	.950	25	rise	25	.490	50	.460
3 a.	48	.960	20	.150	-----	-----	-----	-----
4	8	.205	16	.055	25.6	.018	-----	-----
4 a.	20	.050	42	.020	-----	-----	-----	-----
4 b.	26	.040	30	-----	-----	-----	-----	-----
5	16	.019	-----	-----	-----	-----	-----	-----
5 a.	25	1.250	50	.015	-----	-----	-----	-----
5 b.	22	.037	18	.029	-----	-----	-----	-----

In Case IV in which the blood sugar was at a high level the effect of even a small dose of insulin is quite marked, so that within  $1\frac{1}{2}$  hours after the administration of 8 units of insulin there was a total drop of 148 mg. per 100 cc. In this same case after the blood sugar approached a lower lever, although it was still high, it took  $2\frac{3}{4}$  hours for twice the preceding dose, namely, 16 units, of insulin to produce a drop of 146 mg. per 100 cc., while a third dose of 25.6 units was followed in two hours and fifteen minutes by a fall of only 65 mg. per 100 cc. This and similar results shown in the accompanying table and chart appear to indicate that when the blood sugar is at a high level the insulin produces a greater and a more prompt effect than when the blood sugar is approaching the normal level.

### SUMMARY.

Insulin has no effect on blood sugar in vitro.

A series of 5 cases of diabetes is presented, each of which received large doses of insulin during 24 hours. A fall of the blood sugar concentration occurred after each injection.

When the blood sugar drops to about the level of 50 mg. per 100 cc., in most cases a reaction follows which is manifested by palpitation of the heart, perspiration and weakness. The duration

of this reduction of the blood sugar seems to depend upon the individual case. It would seem, however, that this depends largely on the duration and severity of the disease; i.e., in a case of diabetes of long standing with a low tolerance for carbohydrate, the low blood sugar level which follows the insulin injections will probably not be maintained for more than a few hours at the most.

The size of the dose of insulin required in the 24 hours likewise varies with the severity of the case to which it is given.

Our experience to date does not indicate that there is any uniformity in the reduction of blood sugar after the administration of insulin, although apparently the effect of the insulin bears a direct relation both in time and in the magnitude of its effect to the height of the blood sugar content.

## EXPERIMENTS WITH ILETIN

Table I

Sug. Sol.	Iletin	Let stand Min.	mgm./100 cc. Sugar found
2 cc.	.1	15	857
2 cc.	.1	30	857
2 cc.	.1	45	857
2 cc.	.1	60	857
2 cc.	.1	75	857
2 cc.	.1	90	857
2 cc.	.1	105	857
2 cc.	.1	120	857
2 cc.	.1	135	857
2 cc.	.1	150	857

## EXPERIMENTS WITH ILETIN

Table II

Sug. Sol.	Iletin	Blood	Let stand Min.	mgm./100 cc. Sugar found
2 cc.	.1	.5	15	731
2 cc.	.1	.5	30	731
2 cc.	.1	.5	45	731
2 cc.	.1	.5	60	722
2 cc.	.1	.5	75	697
2 cc.	.1	.5	90	740
2 cc.	.1	.5	105	714
2 cc.	.1	.5	120	681
2 cc.	.1	.5	135	731
2 cc.	.1	.5	150	705

Blood sugar was 89 mgm./100 cc.



EXPERIMENTS WITH ILETIN  
Table III

Sug. Sol.	Iletin	Blood	Let stand Min.	mgm./100 cc. Sugar found
2 cc.	.2	.5	15	758
2 cc.	.2	.5	30	731
2 cc.	.2	.5	45	750
2 cc.	.2	.5	60	714
2 cc.	.2	.5	75	740
2 cc.	.2	.5	90	740
2 cc.	.2	.5	105	714
2 cc.	.2	.5	120	731
2 cc.	.2	.5	135	731
2 cc.	.2	.5	150	714

Blood sugar was 80 mgm./100 cc.



# OBSERVATIONS ON ARTHRITIS DEFORMANS AND CALCIUM METABOLISM.

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The cause and nature of arthritis deformans are recognized as obscure, but abnormal deposits of calcium salts constitute one of the prominent features. At the suggestion of Dr. F. M. Allen, the author made studies of the calcium metabolism in three cases of arthritis deformans, together with analyses of the blood calcium in a series of normal persons and diabetic and nephritic patients.

## METHODS.

The determination of calcium is delicate, and therefore different methods heretofore have given different results. A good review of the older methods is published by de Waard<sup>1</sup>, and recently R. Mayer<sup>2</sup> criticised the new methods, excepting G. W. Clark's method.

The calcium content in the blood does not vary with the sex. Jansen<sup>3</sup> points out, in agreement with French writers<sup>4</sup>, that age influences the calcium level in the blood. Loeper and Bechamp<sup>4</sup> found the difference between old and young persons to be at least 1 mg. per cent. There is physiologically a high calcium content in babies' blood in the first few weeks, which comes down gradually with increasing age. R. Mayer also concludes that the calcium content in whole blood is higher in babies during the first six months, but the serum calcium is constantly about the same as in adults, the average being 11.25 mg. per cent. This difference between whole blood and serum could be explained by a variation in the calcium content of the red cells, which depends upon the surrounding fluid (see Hamburger<sup>5</sup>). Jansen, on the basis of a large series of cases, states that there is no fluctuation of calcium during the day; in other words, ingestion has no influence on the blood calcium concentration, the normal regulation of the body keeping it constant. The experiments of Denis and Minot<sup>6</sup> who fed calcium salts such as the lactate (5 gm. daily through long periods) to men, cats and rabbits, were negative. Clark<sup>7</sup> likewise fed rabbits with calcium-rich diets without affecting the blood calcium. Weiske, Voit and others, experimenting on various animals with calcium-rich and calcium-poor diets, were unable to demonstrate any appreciable difference in the blood calcium. During calcium starvation the level of calcium in the blood remained much the same as in normal animals (Patterson<sup>10</sup>). In taking a blood sample for calcium determination, it is advisable to draw it before breakfast under certain precautions as given by Billigheimer<sup>8</sup>.



Until recently the blood was ashed and afterward the calcium content determined gravimetrically, 100 cc. or more of blood being necessary for one test. Therefore it was an important advance when a few years ago different investigators described methods requiring smaller amounts of blood. Lyman<sup>9</sup> compared the ashing method with his nephelometric method and found negligible differences. De Waard combined the ashing with titration for calcium determination in whole blood. His modification for serum, by merely precipitating the calcium as oxalate, was preferred by many writers. Kramer, Tisdall, and others<sup>10</sup> modified de Waard's method to a micro-method, using 1.0 cc. of blood.

G. W. Clark<sup>11</sup> in 1920 published his method, which avoids ashing. The advantages are the small amount (5 cc. of whole blood) for a test, few reagents, and the precipitation of calcium in the presence of protein. His method was used in our investigation and was found very delicate and satisfactory. A control test was always performed on the same blood and no greater variations than 0.05 cc. were allowed in titration. The principle of his method is: Citrated blood is hemolyzed, the clear blood solution precipitated with oxalate, and the oxalic acid liberated from the precipitate by means of sulphuric acid is titrated, using a micro-burette, against 0.01 N permanganate solution. The range of error is 0.002 mg. calcium in each determination.

NORMAL VALUES.

The normal calcium concentration in the body fluids such as blood, cerebrospinal fluid, etc., is not yet positively defined. The hydrogen ion concentration in blood and tissues is perhaps an important factor in the distribution of calcium. Rabl<sup>12</sup> believes that the solubility of calcium in the blood depends upon the hydrogen ion concentration, and in making any calcium determination the pH has to be considered. If the solution is more acid than a pH of 4.0, calcium oxalate is dissolved. (Shohl<sup>13</sup>.)

TABLE 1

Author	Calcium, mg. %	
	Serum	Whole Blood
Abderhalden . . . . .		11.7
Jansen . . . . .		7.5 - 8.9
R. Mayer . . . . .	10.9 - 12.0	ae
Lyman . . . . .		7.6 - 11.9
Kramer, Tisdall . . . . .	10.0	
Herzfeld-Lubowski (de Waard) . . . . .	10.5 - 12.0	
Zondek . . . . .		10.2 - 10.7
Clark (rabbits) . . . . .		7.0 - 13.0

Table 1, comparing the results by different methods, shows that the figures lie between 7 and 13 mg. per cent. All figures are expressed as calcium.

### CALCIUM METABOLISM.

The important relation between calcium and the parathyroid glands is emphasized by Biedl<sup>14</sup> in a chapter on calcium metabolism. Calcium as an element is an indispensable component of all cells and a good standard for their efficiency. Not only the function and the irritability of the leucocytes, the work of gland, muscle and nerve cells, but also the permeability and irritability of the vessels depend to some extent upon their calcium content. Thus the body requires a certain amount of calcium daily to compensate for catabolized and excreted calcium.

In communities where the water is not calcium-rich, people on a low calcium diet show defects in the development of the whole body. O. Loew<sup>15</sup> in his book on calcium gives an average of 1 gm. calcium as the daily requirement. Sherman<sup>16</sup> in a long series of experiments found that the daily minimum required for equilibrium in normal men was 0.45 gm., but that at least 1 gm. of calcium was necessary for 100 gm. protein food to be utilized. This confirms the finding of Sindler<sup>17</sup> that the calcium balance is highly dependent upon the ingestion of meat. A high protein diet always yields a negative calcium balance. M. H. Givens<sup>18</sup> found by giving a calcium-poor diet, as meat cracker meals, to dogs that they were constantly in a negative calcium balance. Neither sodium bicarbonate nor acid changed this negative balance on a calcium-poor diet. The acid administration increased the urinary excretion of calcium about 200 per cent. He concludes that some of the lime is diverted from the intestinal tract.

The average urinary excretion of calcium by normal adults is between 0.1 and 0.4 gm. daily (expressed as CaO). This is about 10-40 per cent. of the whole amount excreted. The greater part is eliminated by the feces. The proportion is dependent on the calcium content of the food. If the calcium intake is very low, the percentage of the total excretion taking place by way of the kidney is higher, and vice versa. If a rabbit received less than 0.16 gm. calcium per kilogram body weight daily in its food, in the experiments of Goitein<sup>20</sup> there was a steady loss of calcium from the body. Lehmann<sup>21</sup> saw in starvation that the amount of excreted calcium exceeded the amount of the substance in the drinking water. Voit<sup>22</sup> followed the calcium content of different organs on calcium-poor diet and found the bones more brittle and the calcium content of all the organs more or less diminished. Förster<sup>23</sup> showed that the muscles lose 56 per cent. of their calcium on a calcium-poor diet.

### CALCIUM IN METABOLIC DISORDERS.

In parathyroidectomized dogs, according to MacCallum and Voegtlin,<sup>24</sup> there is a remarkable diminution of calcium excretion in the urine and feces.

The calcium balance in diabetics has been found negative (Kahn<sup>25</sup>). As early as 1907, von Noorden found an excretion of calcium much in excess of the quantity in the food. Underhill<sup>26</sup> thought that calcium may play a major role in maintaining the normal equilibrium of the blood sugar regulating mechanism.



Herzfeld and Lubowski<sup>27</sup> recently published a summary of their findings in 70 normal and pathological cases, dividing their results into two groups, one above and one below 11 mg. per cent. calcium in serum.

Zondek<sup>28</sup> states that with functional kidney disorders the normal potassium-calcium ratio in blood is changed. He usually saw hypocalcemia as low as 6 mg. per cent. even in cases without hydremia. In benign hypertension the calcium was normal. R. Mayer<sup>2</sup> reports in the initial stage of rickets slight to pronounced hypercalcemia, which disappeared with convalescence. Hypocalcemia was found in pneumonia by Talbot and Denis.<sup>32</sup> MacCallum and Voegtlin<sup>24</sup> report an abnormally low blood calcium content in parathyroidectomized dogs. Neurath<sup>33</sup> demonstrated hypocalcemia with Wright's method in children with tetany and in parathyroidectomized dogs.

### *Experimental Findings.*

#### *I. Normal Blood Calcium.*

In a series of determinations in five normal members of the staff of this Institute, the calcium concentration of the whole blood was found between 10 and 12 mg. per cent., in agreement with the literature. Figures below 10 mg. may therefore be designated as hypocalcemia, and figures above 12 mg. as hypercalcemia.

One case of acute articular rheumatism showed a normal blood calcium of 10.95 mg. per cent.

#### *II. Diabetics and Nephritics.*

One case of diabetes, symptom-free under treatment, gave a normal figure of 12.1 mg. per cent. calcium. A second treated case of diabetes showed a slight hypercalcemia, namely 14.3 mg. per cent.

Three chronic nephritis patients showed hypocalcemia.

Case 1. Male. Age 40. Blood pressure 248 systolic, 140 diastolic. Marked arteriosclerosis with usual clinical findings. Urine: low fixed specific gravity of 1008-1010. Albumin 3 per mil. Esbach. Microscopically all kinds of hyalin and granular casts, red corpuscles and pus. Calcium 8.42 mg. per cent.

Case 2. Male. Age 42. Blood pressure 235 systolic, 152 diastolic. Pronounced general arteriosclerosis with typical clinical symptoms. Urine: Albumin 1.5 per mil. Esbach. Red blood cells and heavy pus in sediment. Calcium 8.40 mg. per cent.

Case 3. Male. Age 47. Blood pressure 104 systolic, 78 diastolic. Urine: Albumin 2.0 per mil. Esbach. Sediment: few granular and hyalin casts and red blood cells. Calcium 5.88 mg. per cent.



TABLE 2

SUMMARY OF BLOOD CALCIUM OBSERVATIONS IN PATHOLOGIC CONDITIONS

Disease	Calcium Mg. per 100 cc. Whole Blood
Arthritis deformans	
Case 1 . . . . .	20.35
Case 2 . . . . .	18.5
Case 3 . . . . .	27.4
Rheumatic Arthritis . . . . .	10.95
Diabetes melitus	
Case 1 . . . . .	12.1
Case . . . . .	14.3
Chronic nephritis	
a) with hypertension	
Case 1 . . . . .	8.42
Case 2 . . . . .	8.40
b) without hypertension	
Case 3 . . . . .	5.88

*Arthritis Deformans.*

Our three cases showed extreme hypercalcemia (figures above 18.0 mg. per cent., which are the highest mentioned in the literature in pathological cases).

Case 1 was an outside patient, kindly referred by her physician for a single blood examination, as an example of typical advanced arthritis deformans. Calcium 20.35 mg. per cent.

Case 2 was a female, aged 71, seen briefly at the Institute. She has had arthritis for 15 years. Particularly the elbows, wrists, finger joints, and knee joints were swollen, deformed and painful. The head could be rotated only a little to the right. Every available treatment had been tried without benefit. Calcium 18.51 mg. per cent.

Case 3, in which the initial blood calcium was 27.4 mg. per cent., was studied for a time in this Institute, as follows:

Patient No. 1424. Female. American. Age 57. Widow. Housework. Admitted May 27, 1923.

Complaint: Swelling and constant pain in nearly every joint of the body, especially in the left knee. Almost complete fixation of knee joints. Pain on slightest movement. Persistent tinnitus aurium. Insomnia.

Family history negative.

Past history: The usual childhood diseases. Typhoid fever at the age of 22. "Tetanus" at the age of 42. Lobar pneumonia 5 years ago. Appendectomy when 53. Patient always healthy and strong, has done hard manual work scrubbing floors and taking in washing.

Present illness: Patient felt perfectly well until four years ago, when the left wrist began to swell. A few months later both ankles became similarly swollen. At first the joints were not painful, but gradually commenced to be worse and the pain became almost continuous and more

severe. At that time her physician diagnosed chronic arthritis, and after trying various unsuccessful forms of treatment for years, he finally referred her to this Institute.

Physical examination: Height 5 ft. 10 inches. Weight 174 lb. Head and neck apparently normal except stiffness of the back of the neck. Eyes: presbyopia; normal eyegrounds. Oral cavity; teeth false; tonsils atrophic. Cardiovascular system; slight systolic murmur heard at the mitral area, disappears on lying down. Blood pressure 137 systolic, 75 diastolic. Chest normal. Abdomen: post-operative hernia; very lax abdominal walls. Reflexes were not obtained on account of the deformities. Joints: neck stiff; great pain on attempting to rotate neck. Movement of the shoulder joints limited. Elbows fixed in hemiflexion. On account of the great deformities present it was impossible to draw blood in the usual manner; therefore blood was taken from the dorsal veins of the back of the hand. Great deformities of both wrists. The left wrist in particular was fixed in hemiflexion and the bones were irregularly enlarged and nodular. Metatarso-phalangeal joints markedly deformed. Some pain on excursion of hip-joint. Left knee asymmetrically swollen; circumference 21 inches, compared with 17 inches of the other. Patellar tap was obtained. Creaking of both knees. The slightest movement produced severe pain. The skin over the left knee was white, tense and glistening.

Laboratory report. Urine normal. Blood: plasma sugar 131, plasma chloride 614, urea 20, phosphates 4.1, creatinine 2.0 mg. per cent. CO<sub>2</sub> combining power 48.4 volumes per cent. Blood calcium 27.4 mg. per cent. Diagnosis: Arthritis deformans with hydrops in the left knee joint.

#### *"Salt-free" Diet.*

After two and a half weeks on an unweighed chloride-poor diet, the NaCl excretion came down from 3.9 gm. to 0.16 gm. in the 24-hour urine. The plasma chloride was reduced to 519 mg. per cent. (see Table 3 and Fig. 1). The calcium excretion during this period was approximately 430 mg. daily, the average being 225 mg. in urine and 204 mg. in feces. This showed a departure from the usual ratios in the feces and urine. The salt-free diet did not influence the calcium content of the blood. The table shows only slight fluctuation. There was no perceptible improvement, except that the circumference of the swollen left knee was diminished by about two inches, but the patient still complained of great pain in the knee.

#### *Insulin Administration.*

The patient's blood sugar was found in different tests before breakfast to be between 131 and 150 mg. per cent. The carbohydrate assimilation was evidently diminished, as shown by the glucose tolerance test (Table A). This fact Pemberton<sup>28</sup> has often observed in chronic arthritis. The patient's blood sugar went up

TABLE 3  
Case No. 1424. Salt-Free Diet

Date	URINE						BLOOD						
	Vol. cc.	Sp. Gr.	NaCl %	Total NaCl Gm.	CaO Gm.	Glucose	Acet.	Plasma Sugar Mg. %	Plasma NaCl Mg. %	Inorg. P. Mg. %	Urea Mg. %	CO <sub>2</sub> Cap. Vol. %	Calcium Mg. %
May 29	1100	1021	.363	3.9	....	0	0	130	614	4.1	20	48.4	....
31	1300	1014	....	...	....	...	...	...	...	....	..	....	....
June 1	1000	1020	.251	2.5	....	0	0	...	...	....	..	....	27.4
4	700	1029	.161	1.1	.186	0	0	...	...	....	..	....	27.2
10	800	1014	.142	1.1	.220	0	0	139	548	4.9	32	56.2	25.5
13	Insulin started.												
15	1200	1015	.078	.9	.228	0	0	...	...	....	..	....	....
16	950	1013	.082	.8	.185	0	0	150	548	....	36	63.3	....
17	500	1020	.120	.6	.283	0	0	142	536	4.2	..	61.8	....
22	300	1025	.053	.15	.208	0	0	139	519	5.05	20	69.1	24.8



during digestion (Table B). June 18, on a high carbohydrate ration, the blood sugar in the evening was up to 272 mg. per cent., compared with 131 mg. before breakfast. Sugar was never found in the urine.

TABLE A  
GLUCOSE TOLERANCE TEST  
June 10, given 100 gm. glucose to drink with lemon juice

Time	Plasma Sugar mg. %	Urine	
		Vol. cc.	Glucose
Before . . . . .	139	...	0
½ hour . . . . .	214	...	...
1 hour . . . . .	203	160	0
2 hours . . . . .	162	115	0
3 hours . . . . .	128	228	0
4 hours . . . . .	114	350	0

TABLE B  
PLASMA SUGAR (mg. %)

Date	Before Breakfast	During Digestion	
June 12 . . . . .	136	7:00 p. m.	209
June 13 . . . . .	131	7:00 p. m.	195
June 20 . . . . .	142	7:00 p. m.	209
June 23 . . . . .	139	5:00 p. m.	166
		7:00 p. m.	189

The high blood sugar suggested the idea of administering insulin to the patient. Starting at 6 units as a precaution against hypoglycemia, the dosage was gradually increased to 24 units per day (8 units t.i.d.), and the treatment was continued for three weeks. The blood sugar at the end of this period was 116 mg. Insulin was then discontinued, and during the further observation of five weeks the blood sugar remained between 107 and 116 mg. per cent. Throughout the administration of insulin the patient felt somewhat better, and the pain gradually subsided but later returned. No real clinical improvement was apparent from lowering the blood sugar. During insulin treatment combined with salt-free diet no change in the calcium excretion was noted.

TABLE 4  
Case No. 1424. Low Calcium Diet.

Date 1923	Period	URINE					BLOOD					CALCIUM BALANCE					
		Vol. cc.	Sp. gr.	NaCl %	Total NaCl gm.	T. N. gm.	Inorg. P. gm. per L.	Plasma Sugar mg. %	Plasma NaCl mg. %	Blood Urea mg. %	Blood Cal- cium mg. %	Plasma Bicarb. Vol. %	Calcium Expressed as CaO (gm.)				
													Urine	Feces	Total	Intake	Balance
June 24	1 (Neg. balance)	300	1023	.560	1.7	...	0.212	107	560	...	24.82	44.2	0.186	0.051	0.237	0.223	- .014
June 25		650	1025	.708	4.6	9.8	...	...	...	...	...	...	0.375	0.821	1.196	0.189	-1.007
June 26		490	1024	...	...	...	...	112	634	18	...	42.8	0.213	1.374	1.587	0.202	-1.385
June 27		1300	1016	1.008	13.1	10.6	0.202	107	597	20	...	66.2	0.380	0	0.380	0.242	- .138
June 28		1650	1010	.445	7.3	...	...	...	...	...	...	...	0.620	0.434	1.054	0.203	- .851
June 29		1400	1009	.310	4.3	6.3	...	...	...	...	...	...	0.298	0.372	0.670	0.231	- .439
June 30		1800	1010	.494	8.9	8.6	...	...	...	...	...	...	0.419	0.345	0.764	0.196	- .568
July 1		1150	1013	.676	7.6	5.4	...	...	...	...	...	...	0.415	1.702	2.118	0.185	-1.933
July 2		800	1013	.406	3.2	4.9	...	...	...	...	...	...	0.084	0	0.084	0.184	+ .100
July 3		1800	1008	.423	7.6	7.1	...	...	...	...	...	...	0.792	0.091	0.883	0.211	- .672
July 4		1150	1013	.719	8.2	5.9	...	...	...	...	...	...	0.531	0	0.531	0.199	- .332
July 5	2 (Pos. balance)	1600	1013	.527	8.4	7.2	...	...	...	...	...	...	0.189	0	0.189	0.208	+ .019
July 6		800	1010	.503	4.0	4.4	...	116	593	...	...	71.0	0.014	0.177	0.191	0.192	+ .001
July 7		650	1015	.536	3.5	5.7	...	...	...	...	...	...	0.046	0	0.046	0.172	+ .126
July 8		1200	1014	.601	7.2	8.3	...	...	...	...	...	...	0.081	0	0.081	0.188	+ .107
July 9		1250	1013	.828	10.4	8.9	...	...	...	...	...	...	0.061	0.457	0.518	0.192	- .326
July 10	(Insulin discon- tinued)	900	1018	.974	8.7	7.6	...	...	...	...	...	...	0.056	0	0.056	0.197	+ .141
July 11		1550	1013	.671	10.4	8.1	...	...	...	...	...	...	0.019	0.182	0.201	0.191	- .010
July 12		1250	1010	.486	6.1	6.8	...	...	...	...	...	...	0.003	0	0.003	0.212	+ .209
July 13		300	1025	1.001	3.0	3.6	...	110	606	36	17.62	51.9	0.084	0	0.084	0.198	+ .114
July 14		1600	1010	.498	8.0	9.1	...	...	...	...	...	...	0.272	0.219	0.491	0.208	- .283
July 15		1200	1011	.511	6.1	7.2	0.201	...	...	...	...	...	0.180	0	0.180	0.220	+ .040

TABLE 4—Continued

July 16	3 (HCl, 45 drops)	590	1011	.651	3.8	....	....	....	....	....	....	0.218	0.045	0.263	0.240	—	.023
July 17		950	1012	.420	4.0	6.6	....	....	....	....	....	0.178	0.002	0.180	0.214	+	.034
July 18		1300	1012	.655	8.5	7.9	....	....	....	....	....	0.295	0	0.295	0.220	—	.075
July 19		1200	1014	.639	7.7	8.3	....	....	....	....	....	0.275	0.179	0.454	0.212	—	.242
July 20		450	1025	1.020	4.6	5.0	....	....	....	....	....	0.236	0	0.236	0.196	—	.040
July 21	4 (HCl 10 drops)	600	1026	1.029	7.2	7.5	....	....	....	....	....	0.284	0	0.284	0.205	—	.079
July 22		700	1009	.396	2.8	4.6	....	....	....	....	....	0.116	1.520	1.636	0.206	—	1.430
July 23		1300	1011	.597	7.8	....	....	....	....	....	....	0.348	0	0.348	0.210	—	.138
July 24		1100	1009	.342	3.8	6.4	....	....	....	....	....	0.138	0.986	1.124	0.206	—	.918
July 25	5 (Sod. bicarb.)	900	1010	.490	4.4	4.5	....	109	577	10	19.7	0.250	0	0.250	0.201	—	.049
July 26		950	1016	.310	2.5	5.5	....	....	....	....	....	0.203	0	0.203	0.193	—	.101
July 27		1100	1016	.453	5.0	5.8	....	113	....	....	....	0.334	0	0.344	0.187	—	.157
July 28		800	1021	.647	5.2	....	....	....	....	....	....	0.292	1.399	1.691	0.210	—	1.481
July 29		1400	1019	.474	6.6	7.5	....	....	....	....	....	0.329	0	0.329	0.223	—	.106
July 30		1450	1013	.494	7.7	6.3	....	....	....	....	....	0.634	0	0.634	0.191	—	.443
July 31		700	1011	.185	1.3	....	....	....	....	....	....	0.187	2.002	2.189	0.204	—	1.985
Aug. 1		2400	1012	.289	6.9	14.5	....	....	....	....	....	0.618	0	0.618	0.200	—	.418
Aug. 2	6 (Sod. Salicyl.)	1050	1016	.210	2.2	5.3	....	107	544	10	20.1	0.930	0	0.930	0.209	—	.721
Aug. 3		700	1020	.740	5.2	5.8	....	....	....	....	....	0.413	0	0.413	0.193	—	.220
Aug. 4		1100	1020	.720	7.9	8.1	....	....	....	....	....	0.391	0	0.391	0.202	—	.189
Aug. 5		950	1017	.632	6.0	6.7	....	....	....	....	....	0.396	0	0.396	0.213	—	.183
Aug. 6		350	1023	.696	2.4	3.7	....	....	....	....	....	0.186	0.420	0.606	0.178	—	.428
Aug. 7		650	1016	.618	4.0	5.6	....	....	....	....	....	0.356	0	0.356	0.189	—	.167
Aug. 8		400	1019	.498	2.0	4.0	....	....	....	....	....	0.234	0	0.234	0.198	—	.036
Aug. 9		1300	1012	.470	6.1	7.3	....	....	....	....	20.4	0.399	0	0.399	0.212	—	.187



*Calcium-poor Diet.*

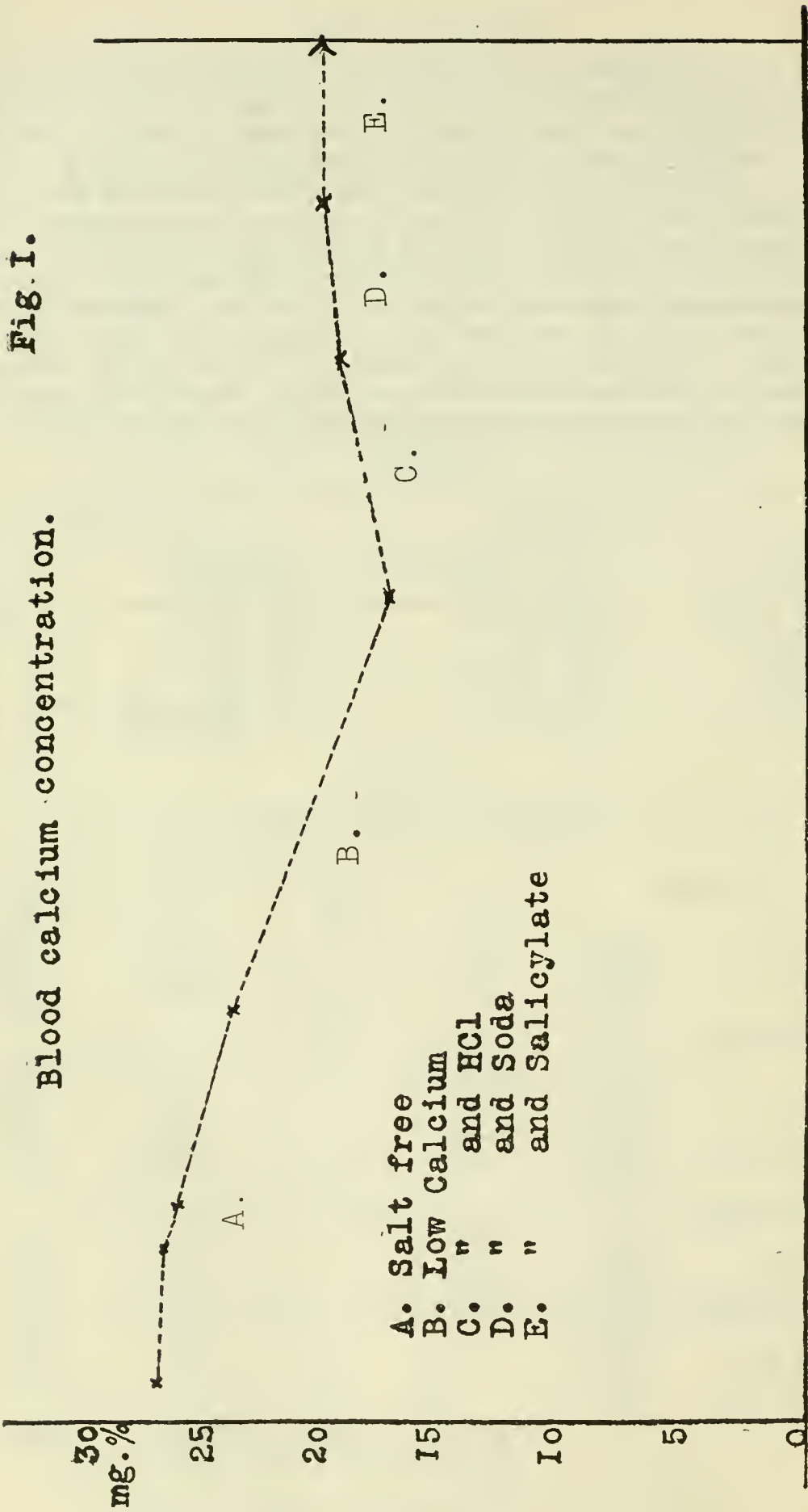
After the foregoing control periods, the patient was placed on a calcium-poor diet in the attempt to reduce the hypercalcemia. We calculated our charts from the food values given by Friedenwald,<sup>29</sup> and arranged the diet with an average daily calcium intake of 0.2 gm., which is 5 to 10 times lower than in a normal diet. As our water contains high calcium in inorganic form as phosphates, carbonates, and sulphates, we gave distilled water to drink. Table 5A shows foods poor in calcium, table 5B those rich in calcium, reckoned as calcium oxide in per cent. of the ash. Foods of higher value than 0.025 gm. per cent. were not used in the diets. One of the daily diet charts for this patient is given as an example (Table 6). The food which the patient did not eat was returned to the diet kitchen, weighed, and deducted from the calculation.

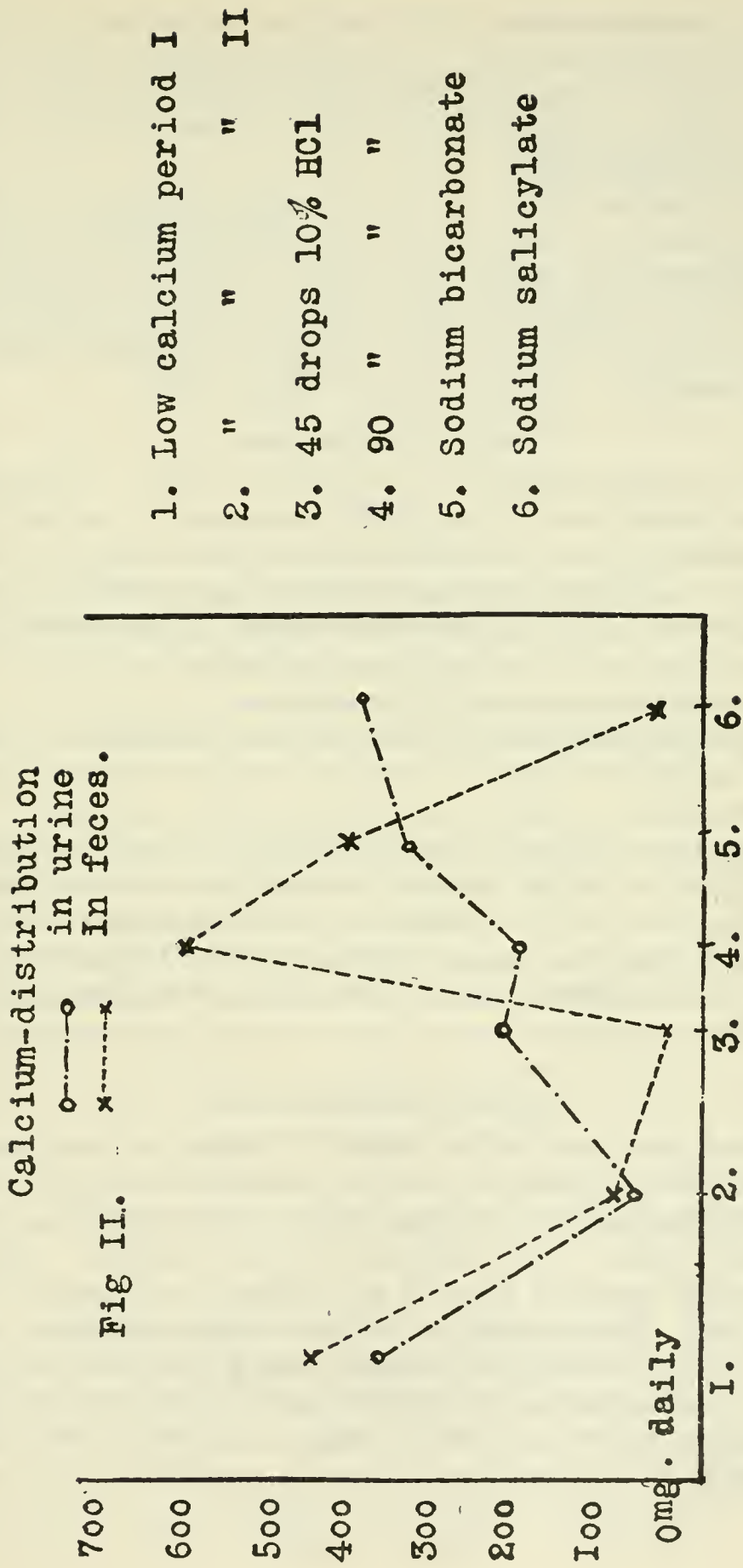
TABLE 5

Low Calcium		High Calcium			
Apple.....	.011	Lean Loin Pork....	.012	Beans.....	.215
Bananas.....	.009	Lean Steak.....	.011	Cabbage.....	.058
Beets.....	.019	Peaches.....	.015	Carrots.....	.077
Bread, white.....	.021	Potatoes.....	.016	Cheese.....	.240
Butter.....	.022	Rice.....	.012	Chocolate.....	.141
Chicken.....	.015	Salmon.....	.011	Cream.....	.147
Egg white.....	.015	Tomatoes.....	.019	Eggs.....	.100
Honey.....	.005	Veal.....	.016	Milk, whole.....	.172
				Peas, dried.....	.137

TABLE 6  
DIET CHART. CASE NO. 1424

July 5, 1923	CALORIES—1903			
	Prot. Gm.	Fat Gm.	C. H. Gm.	CaO Gm.
Rice . . . . 40 gm.	3.2	....	31.0	.0048
Bananas . . . 100 gm.	1.0	....	22.0	.0090
Bread . . . . 50 gm.	5.0	0.5	27.5	.0105
Butter . . . . 30 gm.	....	25.3	....	.0066
Distilled water.				
Veal . . . . 50 gm.	10.5	2.0	....	.0080
Beets . . . . 80 gm.	1.6	....	5.6	.0152
Potatoes . . . 200 gm.	6.0	....	42.0	.0320
Bread . . . . 50 gm.	5.0	0.5	27.5	.0105
Canned pears . 100 gm.	1.0	....	11.0	.0080
Butter . . . . 30 gm.	....	25.3	....	.0066
Distilled water.				
Veal . . . . 50 gm.	10.5	2.0	....	.0080
Tomatoes . . . 100 gm.	1.0	....	4.0	.0190
Egg White . . . 30 gm.	3.9	....	....	.0045
Mashed potatoes 200 gm.	6.0	....	42.0	.0320
Bread . . . . 50 gm.	5.0	0.5	27.0	.0105
Baked apple. . 150 gm.	1.5	....	30.0	.0165
Butter . . . . 30 gm.	....	25.3	....	.0066
Distilled water.				
	61.2	82.0	230.1	0.2083







Daily calcium analyses of the feces and urine were performed by MacCrudden's methods. The calcium balance was then reckoned as the difference between total intake and total output. Graph I shows the distribution of the excretion in urine and feces during the investigation.

For the first period of 11 days on a low calcium diet there was a negative balance. After this time the calcium balance approached an equilibrium, but toward the close of the second period of 11 days it became slightly positive. At the end of this time the blood calcium was diminished to 17.6 mg. per cent., compared with 27.4 mg. on admission. See Table 4 and Figs. 1 and 2.

#### *Acid Administration.*

As the acid-base balance in the blood is said to influence its calcium content, and as the urinary excretion of calcium should be increased by acid administration, the patient for a period of five days was given 15 drops of 10 per cent. HCl t.i.d., which increased the urinary calcium and caused a great disproportion between urine and feces, as shown in Table 3 and Fig. 2. The calcium balance became slightly negative.

For the following five days we increased the amount of hydrochloric acid to 30 drops t.i.d., and the calcium excretion was further increased.

During the acid administration the calcium content of the blood rose slightly to 19.7 mg. per cent., possibly on account of mobilisation of calcium from the tissues into the blood stream. The blood chemistry otherwise was not affected by the hydrochloric acid, except that the plasma alkali fell from 53 to 44.6 volumes per cent. The clinical condition was no better and perhaps became slightly worse.

#### *Alkali Administration.*

Alkalinity of the diet is known to reduce the absorption of calcium, or to increase the proportion excreted through the bowel. Also alkalies (sodium salicylate, sodium bicarbonate) have held a prominent place in the treatment of joint troubles. Therefore this patient was next given 5 gm. sodium bicarbonate t.i.d. for eight days. Table 4 shows a continued negative balance, though the excretion was slightly smaller than during the acid period. The calcium content of the blood remained practically unchanged. The plasma bicarbonate was increased from 44.6 to 65.3 volumes per cent.

During the next period of seven days the patient was given a high dosage of sodium salicylate (25 grains t.i.d.). The calcium excretion was diminished but the balance was still negative. The diminution in the excretion through this last period could be explained by an obstinate constipation. The strongest cathartics gave only temporary relief.

During the whole period of the calcium-poor diet the patient felt somewhat improved. Objectively it was seen that she rested in the night without continuous pain, and also that the pain in the left knee was much diminished. The symptomatic benefit, however, seemed to be sufficiently explained by hospitalization, and there appeared to be no decisive or fundamental benefit from the diet.

#### *Summary.*

1. In agreement with other writers, we find 10.0-12.0 mg. per cent. as the normal value for calcium in whole blood.

2. In diabetes under treatment we found normal to slightly increased values for the blood calcium.

3. Hypocalcemia was present in three cases of chronic kidney disorders. We made no study of the possible significance of this phenomenon.

4. Remarkable hypercalcemia was found in three cases of arthritis deformans (18.5 to 27.4 mg. per cent).

5. In one case of arthritis deformans studied in the Institute, the following observations were made:

- (a) Administration of insulin reduced the existing hyperglycemia but made no change in the calcium metabolism or the clinical condition.

- (b) Rigid limitation of sodium chloride intake reduced the chloride content of the blood and urine, but had no significant influence on the calcium analyses or the clinical condition.

- (c) A calcium-poor diet reduced the blood calcium, but not to normal. This stubborn persistence of the hypercalcemia was striking.

- (d) Administration of acid, alkali or salicylate changed the ratio of calcium excretion in urine and feces, but did not alter the slight negative balance on the calcium-poor diet.

If these findings can be confirmed in a larger series of cases, they seem to indicate a marked disorder of calcium metabolism in arthritis deformans. It is not impossible that a calcium-poor diet may have some benefit in the early stages, but it brought no improvement in the advanced case which was studied.

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# CATABOLISM OF ODD IN COMPARISON WITH EVEN CARBON FATTY ACIDS IN MAN

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The important investigations of F. Knoop<sup>1</sup> and further of Embden<sup>2</sup> have shown that aliphatic and aromatic saturated fatty acids in their catabolism in the body are oxidized at the  $\beta$ -carbon atom; the  $\alpha$ -carbon atom, together with the carboxyl group, are thereby split off. Thus a loss of two carbon atoms at once always takes place.

Normal fatty acids with an *even* number of carbon atoms thus finally yield butyric acid, which can be easily oxidized to  $\beta$ -hydroxybutyric acid or acetoacetic acid, and this in turn is readily convertible into acetone. As natural fats are glycerides of fatty acids with an even number of carbon atoms, it is therefore possible that under abnormal circumstances the butyric acid formed, instead of finally liberating carbon dioxide and water, will produce the ketone substances of acidosis.

A fatty acid with an *odd* number of carbon atoms, by the same kind of breakdown, can never directly yield butyric acid or the acetone bodies. It has also been shown<sup>3</sup> that only normal fatty acids with an even number of carbon atoms produce acetone bodies.

The experiments of Blum and Woring<sup>4</sup> have shown that when propionic acid is given to normal dogs and rabbits, lactic acid and pyruvic acid are excreted, which must be regarded as a case of  $\alpha$ -oxidation of propionic acid. "But since normal  $\beta$ -oxidation with formation of ketonic acid is no longer possible with a three-carbon acid it need not be regarded as violating the  $\beta$ -oxidation rule applicable to acids containing four or more carbon atoms. It appears, therefore, that in the metabolism of normal fatty acids containing four or more carbon atoms acetoacetic acid is a common metabolite of all those with an even number of carbon atoms, while lactic acid is common to those with an uneven number."<sup>5</sup>

Ringer<sup>6</sup> found that valerianic and heptylic acids gave glucose in the diabetic organism in proportion to the amount of propionic acid they might yield through  $\beta$ -oxidation.

Kahn<sup>7</sup> has recently succeeded in preparing an odd carbon fatty acid fat. The methods of preparation were as follows:

"Stearic acid has its acid group substituted by an organic radical and upon oxidation with a strong oxidizing mixture the  $C_{17}$  acid ( $C_{16}H_{33}COOH$ , margaric acid) is produced. This is easily purified and then united with glycerol to form a neutral fat."

"This fat, when well prepared, is of a white creamy color, odorless and tasteless, melting at  $38^{\circ}$  C. and neutral in reaction. When cold and granulated it is quite palatable. It is absorbed to the extent of about 90 per cent., is catabolized in the body and does not yield the ketone substances derived from butyric acid. The diabetic patient's diet can be much increased by this fat. It allays hunger and stops the loss of weight."

The main problem in this investigation was to find the effect of this odd fat on the ketosis\* induced in a normal individual (the writer, himself, who was in perfect physical condition, twenty-seven years old, born in Sweden, 5 feet 9 inches tall and weighing 165 pounds), and to follow the metabolism of this odd fat and natural fat under special conditions.

After seven days (period I) on a diet of 20 gm. carbohydrate, 100 gm. protein and 177 gm. natural fat (2,073 calories), the complete 24 hour urine specimen showed 3.3 gm. of acetone bodies. 100 gm. of the natural fat was then replaced by 100 gm. of odd fat, and the acetone bodies in four days (period II) dropped to 1.5 gm. in the 24 hour urine. After replacement of the odd fat by 100 gm. of natural fat, i. e., the same diet as during the first seven days, the acetone bodies in two days (period III) increased to 4.7 gm. With the same protein-fat ration, the carbohydrate was increased to 70 gm. per day (2,273 calories). The acetone bodies then disappeared in less than four days (period IV).

During the course of the experiment we can therefore distinguish the following diet periods:

Periods	CH. gm.	Fat gm.		Protein gm.	Calories
		Natural	Odd		
Period I, 7 days Nov. 12-18	20	177	0	100	2073
Period II, 4 " " 19-22	20	77	100	100	2073
Period III, 2 " " 23-24	20	177	0	100	2073
Period IV, 4 " " 25-28	70	177	0	100	2273
Period V, 1 day " 29	Un-weighed	Un-weighed	0	Un-weighed	.....

Water was taken during the whole experiment as desired.

Table I shows some of the results obtained.

\* Kahn has performed a similar experiment, but when this investigation began his results were not yet published. (American Jour. Med. Sciences, 166, 1923, 833.)

TABLE I  
24-hour urine specimen

Date 1923	Urine				Blood Plasma		Body Wgt. kg.	
	Vol. cc.	Sp. gr.	Total Ace- tone gm.	NH <sub>3</sub> gm.	CO <sub>2</sub> Vol. %	Glu- cose %		
Nov. 11	-----	-----	0	-----	55	0.11	75.0	
Nov. 12	1530	1.014	Traces	0.8	---	-----	75.0	<i>Period I.</i> Natural fat 177 gm. CH. 20 " Protein 100 "
13	1700	1.010	1.7	1.3	---	-----	-----	
14	1800	1.015	-----	1.4	---	-----	-----	
15	2050	-----	2.5	1.4	52	0.09	69.5	
16	1200	1.011	3.0	-----	---	-----	-----	
17	-----	1.012	-----	-----	---	-----	-----	
18	1800	1.010	3.3	1.5	---	-----	69.5	
Nov. 19	2200	1.005	4.3	2.0	---	-----	-----	<i>Period II.</i> Natural fat 77 gm. Odd " 100 " Protein 100 " CH. 20 "
20	1700	1.010	2.5	2.8	---	-----	69.0	
21	2150	1.009	2.1	1.8	51	0.11	-----	
22	2500	1.005	1.5	1.4	---	-----	68.5	
Nov. 23	1900	1.005	3.1	1.8	---	-----	-----	
24	1900	1.012	4.7	2.6	---	-----	68.5	<i>Period III.</i> Natural fat 177 gm. CH. 20 " Protein 100 "
Nov. 25	2050	1.009	1.5	1.3	---	-----	-----	<i>Period IV.</i> Natural fat 177 gm. CH. 70 " Protein 100 "
26	1600	1.010	0.6	1.5	---	-----	-----	
27	2150	1.008	Traces	1.1	---	-----	68.5	
28	1600	1.010	0	0.7	---	-----	-----	
Nov. 29	1500	-----	0	0.7	---	-----	68.5	<i>Period V.</i> Unweighed diet.

The plasma never showed more than a faint qualitative reaction with nitroprusside.

Concerning the calculation of the acetone bodies, see p. 154.

As the writer during the whole experiment was doing his usual work the diet, 2,073 calories, was too low and he therefore felt weak, especially during the first part of period I and during the whole odd fat period II. As is shown below, the odd fat was absorbed to about 80%; therefore the total calories during period II amounted to only about 1,900. The odd fat was much more disagreeable to take than the natural fat (butter). It had a disagreeable odor and taste. It was taken as a warm drink. Its effect was slightly nauseating. During period II, there was no more loss of body weight, and the odd fat therefore seemed to be utilized.



*The absorption of odd fat in the body.*

As part of the odd fat was not absorbed (see below) and appeared in the feces in the form of large solid pieces, it was often impossible to get a homogeneous feces mixture and much less possible to take out a representative part for the common fat determination. In these cases the whole feces sample was mixed with water and the odd fat was strained out through a fine wire sieve, and then thoroughly washed, dried and weighed. The isolated substance consisted of fatty acids, which to about 90-95 per cent. were united with glycerol as neutral fat. The remaining 5-10 per cent. was free acids; no soaps could be found.

In this way 15-20 per cent. of the whole odd fat could be found in the feces. In this determination, if emulsified fat (and soap) was present, it naturally was not estimated. In other cases, where the consistency of the feces was of such a nature that a homogeneous feces mixture was obtainable, we also found 15-20 per cent. fat\* (using the common fat methods).

Accordingly, the odd fat was absorbed to about 80-85 per cent. The unabsorbed part passed through the bowels without undergoing any change. Kahn found an absorption of about 90 per cent. The absorption of natural fat during periods I and III amounted to about 95%.

Table I shows that the sugar and carbon dioxide content of the blood during the whole experiment did not undergo any appreciable change.

During the first days of period I there was a marked decrease in body weight, probably due to loss of water. During the remaining time of the experiment the body weight was practically constant.

### ORGANIC ACIDS

#### 1. Acetone bodies

The acetone bodies were determined by Van Slyke's method.

a. *Total acetone bodies.* In Table II, column 5, the acetone bodies are calculated as grams of acetone in the total 24-hour urine specimen, on the assumption that the molecular proportion of these substances in the form of  $\beta$ -hydroxybutyric acid is 75% of the total, which proportion is usually approximated in acetonuria.\*\*

In column 4 the acetone bodies are calculated as cc. of 1-M concentration in the 24 hour urine. These values are graphically shown in figure 1 (as a function of the experimental time). Figure 1 also shows

\* In these figures unabsorbed parts of the natural fat taken are included.

\*\* Van Slyke, Journ. biol. Chem., 32, 1917, 455. Hawk, Physiological chemistry, seventh edition, p. 553. "Because  $\beta$ -hydroxybutyric acid yields only 0.75 molecule of acetone, the calculation is strictly accurate only when this proportion is present, but the error introduced by the use of the calculation above is for ordinary purposes not serious. The actual errors in percentage of the amounts determined are as follows: Molecular proportion of acetone bodies as  $\beta$ -acid, 0.50, error 6.5%;  $\beta$ -acid 0.80, error 1.3%."

volatile acids, total organic acids and the acidity of the urine (obtained by direct titration using phenolphthalein as indicator) as cc. of 1-N acids in the whole urine volume as a function of the experimental time. All the substances shown in figure 1, therefore, are expressed in comparable values.

The curve for the acetone bodies shows that these substances, during *period I* (177 gm. natural fat, 20 gm. CH. and 100 gm. protein), *regularly*

TABLE II  
Total acidity with proportions of component organic acids.  
24-hour urine specimens

	1	2	3	4	5	6	7	
Date 1923	Acidity by Titration; cc. of 1-N Acids	Cc. of 1-N Org. Acids (Total)	Cc. of 1-N Vola- tile Acids	Cc. of 1-M Ace- tone Bodies	Gm. of Ace- tone Bodies	$\beta$ -Hydroxy- butyric Acid	Gm. of Ammonia	
Nov. 11	---	---	-----	0	0	---	-----	
11-12	---	---	-----	---	Traces	---	0.8	<i>Period I.</i> Natural fat 177 gm. CH. 20 " Protein 100 "
13	---	---	-----	29	1.7	---	1.3	
14	45	---	-----	---	---	---	1.4	
15	41	---	-----	43	2.5	---	1.4	
16	---	---	-----	52	3.0	---	---	
17	---	---	-----	---	---	---	---	
18	32	---	-----	57	3.3	---	1.5	
19	9	---	-----	70	4.3	+	2.0	<i>Period II.</i> Natural fat 77 gm. Odd " 100 " CH. 20 " Protein 100 "
20	18	28	10.2	43	2.5	+	2.8	
21	34	30	6.3	36	2.1	Traces	1.8	
22	21	35	7.0	26	1.5	0	1.4	
23	46	18	6.2	53	3.1	+	1.8	<i>Period III.</i> Natural fat 177 gm. CH. 20 " Protein 100 "
24	41	41	5.4	81	4.7	+	2.6	
25	52	22	8.0	26	1.5	0	1.3	<i>Period IV.</i> Natural fat 177 gm. CH. 70 " Protein 100 "
26	26	15	8.1	10	0.6	0	1.5	
27	27	---	11.5	---	Traces	0	1.1	
28	29	---	9.5	0	0	0	0.7	
29	28	15	8.5	0	0	0	0.7	<i>Period V.</i> Unweighed diet.

The figures for the organic acids (column 2) do not include acetoacetic acid.

*increase* and at the end of this period (November 18) reach a maximum value of about 60 cc. of 1-M acetone bodies. During *period II* (100 gm. odd fat, 77 gm. natural fat, 20 gm. CH. and 100 gm. protein) the acetone

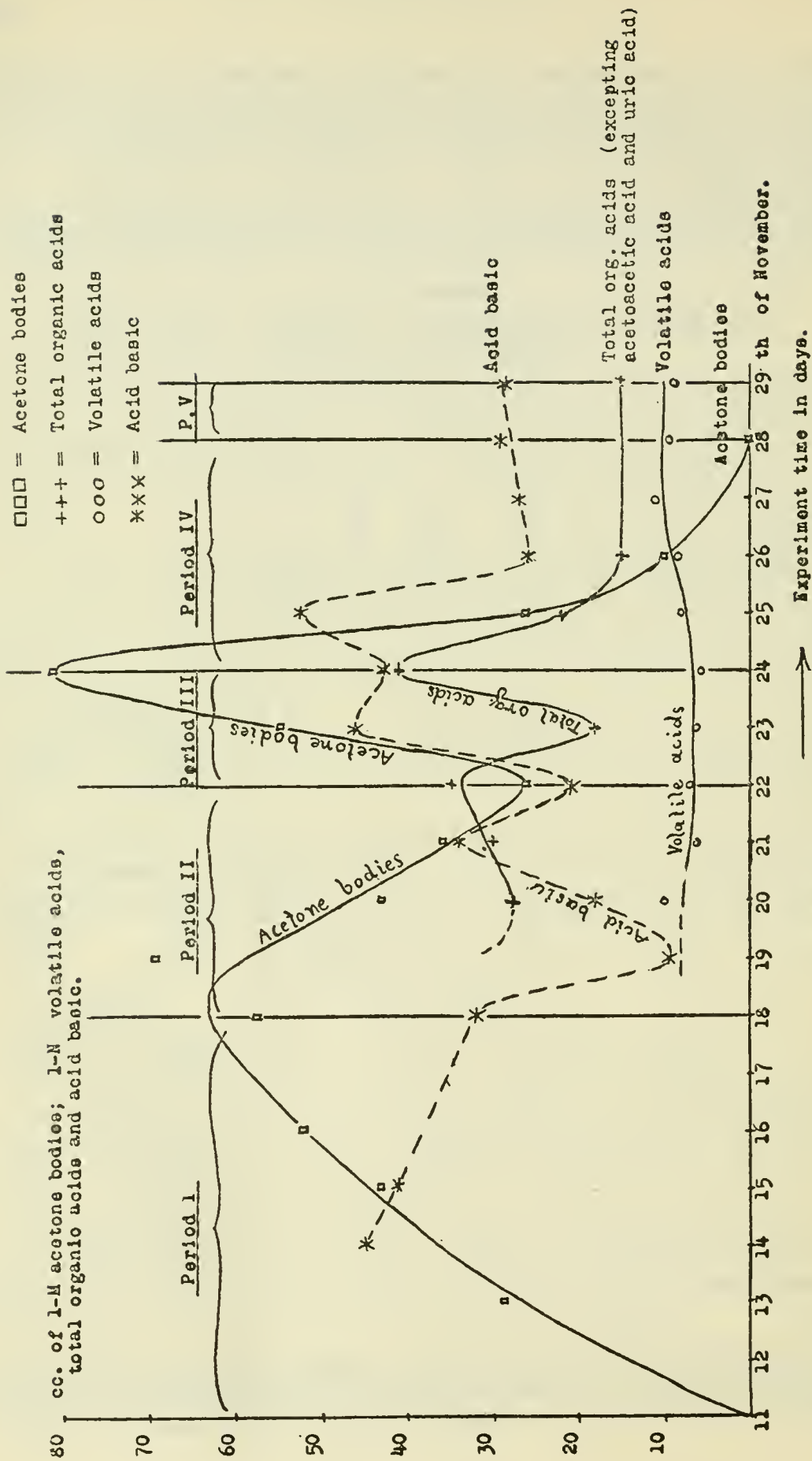


Fig. 1.



bodies *decrease rapidly*. On the last day of this period (November 22) a minimum value of 26 cc. (1-N concentration) was excreted. *In period III*, the odd fat was replaced with natural fat and the acetone bodies within two days *increased* to 81 cc. During *period IV* (177 gm. natural fat, 70 gm. CH. and 100 gm. protein) the increased carbohydrate intake produced a *gradual disappearance* of the acetone bodies to a negative reaction.

As natural fat was utilized nearly completely, but odd fat only to about 80% (see above), a smaller amount of fat ( $77 + \frac{80.100}{100} = 157$  gm.) during period II was absorbed than during the periods I and III (177 gm.). But the difference is far too small to account for the observed rapid decrease in the acetone bodies during period II. The conclusion, therefore, must be held, that odd fat in its catabolism in the human body forms none, or at least a much smaller amount of acetone bodies than natural fat. This corresponds wholly with Kahn's results.

b.  *$\beta$ -hydroxybutyric acid*. The  $\beta$ -hydroxybutyric acid was separately determined in the 24-hour urine by Van Slyke's method (after the acetoacetic acid and acetone had been boiled off<sup>8</sup>). On the 20th, 21st, 23rd and 24th small amounts were found, but as the method for such small quantities is not very accurate, no quantitative figures have been given in Table II, column 6. The results show that the  $\beta$ -acid during the *odd fat period II decreased* and had disappeared with the minimum point of the acetone curve on the 22nd. When this curve subsequently *mounted* (period III), the acid again appeared.

c. *Relation between  $\beta$ -hydroxybutyric acid and total acetone bodies*. The assumption that with acetonuria the  $\beta$ -hydroxybutyric acid should be 75% of the total acetone bodies is in this case not always true. For example, on the 22nd, 25th, 26th and 27th there was no  $\beta$ -hydroxybutyric acid in the urine, but still an appreciable amount of acetone or acetoacetic acid or both.\*<sup>9</sup>

Therefore, on the 22nd, 25th, 26th and 27th, when no  $\beta$ -acid was present, the figures for the acetone bodies in Tables I and II and in figure 1 are 20% too high (compare with the calculation of p. 154 and the note on the same page. One molecule  $\beta$ -acid yields only 0.75 molecule acetone).

\* Kennaway showed that in cases of human ketosis, when the total daily excretion of acetoacetic and  $\beta$ -hydroxybutyric acids exceeds 2.5 gm., the ratio of the two acids is in the proportion of 2 to 5 molecules of the hydroxy acid to one of acetoacetic acid. *Biochem. Jour.* VIII, 1914, 355. That the relation of the two acids is capable of considerable variation as a result of changed conditions, follows from experiments on rabbits by Blum and Nakano. *Compt. rend: Soc. de Biol.*, 1919, LXXXII, 1435. Dakin, "Physiological oxidations," *Physiol. Rev.* 1, 1921, 406.

### *Summary of the Acetone Bodies*

1. In a normal man with absence or insufficiency of carbohydrate, acidosis is produced by a metabolic breakdown of natural fats (which are all glycerides of fatty acids containing an even number of carbon atoms), yielding butyric acid and acetone bodies.

2. Odd carbon fatty acid fat in its catabolism in the human body, in the absence of carbohydrates, or if they be given in insufficient quantities, forms no acetone bodies.

3. Acidosis produced in a normal man by a combined high natural fat (177 gm.) and low carbohydrate (20 gm.) diet can be cleared up in a few days by replacing 100 gm. of the natural fat with 100 gm. of odd carbon fatty acid fat, or by increasing the quantity of carbohydrate to 70 gm. per day (fat diet unchanged).

4. With small amounts of acetone bodies in the urine,  $\beta$ -hydroxybutyric acid could not be isolated among the excreted acetoacetic acid and acetone. It was only when the acetone bodies increased that  $\beta$ -hydroxybutyric acid appeared.

### *2. Volatile Acids*

*Determination.* 300 cc. of the 24-hour urine, after adding 5-10% concentrated sulphuric acid, was distilled with steam. The distillation was continued until the steam had no further acid reaction and the volume of the distillate was about 300 cc. The total amount of the volatile acids was determined by titration of an aliquot part of the distillate with N/10 sodium hydroxide (phenolphthalein). From the value so obtained the total quantity of volatile acids in the whole 24-hour urine sample was calculated as cc. of 1-N acid (see Table II, column 3).

In steam distillation of acid urine the distillate may contain lower monobasic fatty acids such as formic, acetic, propionic and butyric acids. The hippuric acid in the urine, on heating with sulphuric acid, will be split into glycocholic and benzoic acid. The latter is volatile and, therefore, will appear in the distillate. The phenols in the urine are also carried over to the distillate.

If the urine contains lactic acid, small amounts of this may appear in the distillates. A. Partheil<sup>10</sup> and F. Utz<sup>11</sup> have shown that lactic acid is volatile, though to a very small degree. The presence of acetic acid makes the lactic acid more volatile. E. B. Hart and I. I. Williams<sup>12</sup> showed that lactic acid with steam at 100° C. is volatile only to a very slight extent. By heating with dilute sulphuric acid, lactic acid, like many other  $\alpha$ -hydroxy acids, will be split to acetaldehyde and formic

acid.\* Therefore, the presence of formic acid in the distillates may be in part due to lactic acid. The acetoacetic acid by the distillation will be transformed into acetone and carbon dioxide.

$\beta$ -hydroxybutyric acid, like all the  $\beta$ -hydroxy acids, easily splits off  $H_2O$  and thereby gives an unsaturated acid (for  $\beta$ -hydroxybutyric acid, crotonic acid). If the distilling urine is acid enough, such a reaction will take place, and the volatile crotonic acid formed will appear in the distillate. In our experiment, as is shown below, such a reaction did not take place.

The total amounts of volatile acids are graphically represented in figure 1, as cc. of 1-N acids in the total urine volume. The abscissa shows the time of the experiment in days. During the odd fat period (II) there was the same quantity of volatile acids in the urine as during the normal fat period (III). With the increase of carbohydrate from 20 to 70 gm. per day, one can see a slight increase (compare period III and IV) in the amount of volatile acids.

#### *Tests for different acids.*

a. *Formic acid.* A few cc. of the distillates were treated in a test tube with an ammonia-silver nitrate solution and heated. If formic acid is present, a black precipitate of reduced metallic silver will be formed with evolution of carbon dioxide.

All the distillates showed the same faint positive reaction of formic acid.

b. *Acetic acid.* A part of the thoroughly neutralized distillates was treated with dilute ferric chloride solution. In all distillates there was a negative or only a very faint positive reaction.

c. *Propionic acid.* 200 cc. of each distillate was put into a large beaker and after addition of lead oxide (10-20 gm.) evaporated to dryness over a waterbath.<sup>13</sup> The residue was mixed with cold (15° C.) water, whereby the lead salts of the volatile acids present were dissolved. After filtration the filtrate was heated and a portion evaporated. The basic lead salt of the propionic acid is soluble in cold water, but not in hot (whereas the lead salts of the formic, acetic and butyric acids are soluble both in cold and in hot water) and, therefore, is thrown out of solution by heating. The solution was filtered hot and the whole filter paper was put into an Erlenmeyer flask and after addition of 300 cc. water and 10-15 cc. concentrated sulphuric acid distilled with steam. This distillate, to the amount of 300 cc., was titrated with N/10 sodium hydroxide in order to determine the amount of propionic acid in the urine. Only 2.5 cc. of sodium hydroxide was necessary to produce a neutral reaction.

*During the whole experiment, therefore, none or only a trace of propionic acid had been formed.*

\* With the concentration of sulphuric acid used in this experiment, such a division of lactic acid will take place only to a very small degree.



d. *Phenols*. The distillates were examined with Millon's reagent. They all showed a negative or very faint positive reaction indicating only traces of phenols.

### *Summary of the Volatile Acids*

Under the above conditions:

1. Odd fat, in its breakdown in the body, seems to have the same influence on the quantity of volatile acids in the urine as natural fat.

2. By increasing the carbohydrate from 20 to 70 gm. per day with the same high (177 gm.) fat diet (and normal protein), there was a slight increase in the amount of volatile acids in the urine.

3. The volatile acids in the urine seem, under the same conditions, to undergo no important qualitative or quantitative change with either odd fat or natural fat diet. The main part of the volatile acids seems to consist of formic acid. Acetic acid and propionic acid are not found or only in very small amounts among the volatile acids.

### *3. Total organic acids*

*Determination*. The 24-hour urine sample was thoroughly shaken, so that the precipitate of calcium oxalate, if any, would be equally distributed in the urine. An aliquot part (usually 100 cc.) was measured off and the colloids precipitated to prevent emulsification in the subsequent ether extraction. It was found that these colloids were wholly precipitated by the addition of powdered ammonium sulphate to the point of saturation of the urine (about 120 gm. for 100 cc. urine); a small amount of the undissolved salt should remain. After 12 hours at room temperature the solution was filtered through a dry folded filter, in the bottom of which was a small amount of silicious earth. In this way an absolutely clear filtrate was obtained which did not emulsify with ether. It was then not necessary to use a more complicated precipitation with phosphotungstic acid.

The volume of the filtrate was determined and concentrated sulphuric acid to an amount of 1/20 part of the whole filtrate was added, in order to liberate the organic acids. By this addition of sulphuric acid a precipitate was frequently formed, which was filtered off. In a separating funnel the solution was shaken twice (each time for ten minutes) with at least an equal volume of ethyl ether and allowed to separate. After the urine layer had been drawn off, the ether-acid layer was shaken with a little water and again allowed to separate. An equal volume of alcohol, 90-94%, was added to this washed ether-acid solution and the whole titrated with N/10 alcoholic potassium hydroxide solution, using phenolphthalein as indicator. From the value obtained by titration the

total quantity of organic acids (except the acetoacetic acid and the uric acid) in the whole 24-hour urine volume was calculated as cc. 1-N acid (see Table II, column 2).

As this determination was done when the urine was already several days old, acetoacetic acid, if any was present, had been split into acetone and carbon dioxide. *Thus the above obtained values show all the organic acids except the acetoacetic acid and the uric acid (which is insoluble in ether).*

The graph, figure 1, shows the variations of the total organic acids (as cc. of 1-N acids) as a function of the experimental time. On the 23rd, 24th and 25th (high natural fat diet), the organic acid curve runs parallel with the "acetone bodies" curve. The variations in the organic acids here probably depend upon  $\beta$ -hydroxybutyric acid, which has been formed by this time (on the 23rd and 24th, the urine gave a positive reaction for this acid. See Table II, column 6.). When no more  $\beta$ -acid was formed (25th to 29th), the organic acid quantity became constant.

On the 19th and 20th,  $\beta$ -oxybutyric acid appeared in the urine; on the 21st only a trace was perceptible; on the 22nd it had disappeared (Table II, column 6). Still the organic acid curve rose at this time (during the last half of the odd fat period II) and culminated on the 22nd, at the end of the odd fat period. At the beginning of the natural fat period III, the curve rapidly drops. With the culmination of the organic acid curve on the 22nd, the acetone bodies curve shows a minimum.

This seems to indicate *that the odd fat on its breakdown in the body, under the conditions prevailing in this experiment, forms one or more non-volatile acids, not  $\beta$ -hydroxybutyric acid or acetoacetic acid, for the volatile acid quantity was constant (see above).*

In the described ether extraction all the organic acids present in the urine except uric acid are taken up by the ether. Volatile acids,  $\beta$ -hydroxybutyric acid, acetoacetic acid, pyruvic acid, lactic acid, benzoic acid, citric acid,\* etc., are readily soluble in ether (lactic acid, however, not so readily as the others).

Control extractions were done and the same value for the organic acids was always obtained.

Column 1 in Table II shows the acidity of the urine (as cc. of 1-N acid) obtained by titration with N/10 sodium hydroxide solu-

\* Amberg and MacClure show that this acid occurs in amounts equivalent to 6 to 7 cc. of 1-N acid in a normal 24-hour urine. Van Slyke and Palmer, J. Biol. chem., 41, 1920, 567.

tion. The values are graphically represented in figure 1 (acid-basic curve). A comparison between this curve and the total organic acid curve shows that an acid-basic titration does not well represent the amount of organic acids in the urine.

#### 4. *Lactic Acid and Pyruvic Acid*

The organic acid curve shows that there must have been a formation during the odd fat period II of some non-volatile acids, which are not  $\beta$ -hydroxybutyric acid or acetoacetic acid. It seemed advisable to examine the urine for lactic acid and pyruvic acid. The methods used and the results obtained are described below.

##### *Determination.*

Lactic acid is not so readily soluble in ethyl ether as other organic acids, and in Embden's laboratory it has been found that an extraction with *Lindt's* apparatus for from 24 to 48 hours is necessary to remove lactic acid completely from an aqueous solution. *Ohlsson*,<sup>14</sup> therefore, used amylic alcohol which more readily takes up lactic acid than ether. The principle in *Ohlsson's* method is the following: The aqueous solution of lactic acid is saturated with ammonium sulphate to precipitate emulsifying colloids. By shaking with amylic alcohol the lactic acid is extracted and the amylic alcohol solution is then shaken with an equal volume of 2% sodium carbonate. From this carbonate solution the absorbed amylic alcohol, which will interfere with the subsequent determination of lactic acid, is removed by treating with benzol.

Fürth and Lieben,<sup>15</sup> however, found that all amylic alcohol cannot be removed from the carbonate solution with benzol. They found that if a current of steam was blown through the boiling carbonate solution, which had been previously neutralized, all amylic alcohol was carried over. As free lactic acid is volatile to a small extent, it is therefore necessary to avoid an acid reaction, which would cause a loss of lactic acid. On the other hand, if the solution is alkaline, the lactic acid can be destroyed by long heating. The neutralization, therefore, must be done carefully.

The urine specimens were all made sterile by adding sulphuric acid (concentrated acid to the amount of 1/20 part of the specimen volume). As the urines had to stand a long time before the determination of lactic acid could be done, it is possible that part of the free lactic acid during this time had formed a lactid compound, which is not transferable from the amylic alcohol to the carbonate solution. At the time of examination, therefore, they were neutralized with solid sodium hydroxide to a slight alkaline reaction. In order to precipitate colloids which would emulsify with amylic alcohol, the ammonium sulphate precipitation used by *Ohlsson*, and Fürth and Lieben (described on page 160) was found convenient



(phosphotungstic acid, used by Genia Riesenfeld,<sup>16</sup> was not necessary). With this precipitation, the urine specimen (neutralized as above and saturated with ammonium sulphate) had to stand for at least 12 hours at a slightly alkaline reaction. During this time a lactid compound, if there were any, would be converted by the slight alkalinity to the normal acid salt. The solutions were filtered as described on page 160 and the volume of the total filtrate was determined.

An aliquot part (usually 100 to 150 cc.) of the filtrate was transferred to a separating funnel, and concentrated sulphuric acid to the amount of 1/20 part of the volume was added. Then 150 to 200 cc. amylic alcohol was poured into the funnel and this shaken for five minutes. After separation the aqueous layer was drawn into a beaker, and in another funnel the amylic alcohol was shaken for five minutes with an equal volume of sodium carbonate solution (2%). After separation the carbonate solution was drawn off and the amylic alcohol in the first funnel was shaken again for five minutes with the ammonium sulphate urine, etc. This procedure was repeated five times. It is necessary to take care that none of the acid urine is carried over to the carbonate solution with the amylic alcohol, thus neutralizing the carbonate.

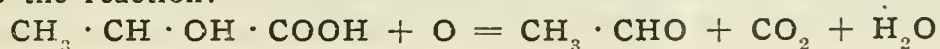
To the sodium carbonate solution, which had taken up all the lactic acid, a few drops of methylred indicator was added and the solution carefully neutralized with sulphuric acid (if pyruvic acid was to be determined at the same time, hydrochloric acid was used. See below). In a flask, the solution was heated to boiling and for about 1/2-1 hour a current of steam was blown through. As carbon dioxide from the sodium carbonate and the sulphuric acid escaped, the solution became alkaline and more acid was added. In this way all traces of amylic alcohol are freed from the solution.

If only lactic acid was to be determined, the solution would be transferred to a distilling flask of the Fürth Charnass apparatus for determination of lactic acid and treated as below.

If, however, lactic acid and pyruvic acid were to be determined, water and concentrated hydrochloric acid (85 cc.) were added to the solution, so that a volume of 400 cc. was obtained and the hydrochloric acid content became 10%.<sup>17</sup> 5 gm. zinc powder was added, and by heating over a water bath with a reflux condenser for about one hour pyruvic acid, if present, was reduced to lactic acid.<sup>17</sup> Undissolved zinc was filtered off, the filtrate neutralized with solid sodium carbonate and transferred to the distilling flask of the Fürth Charnass apparatus for determination of lactic acid. Water was here added to make a total volume of 600 cc., and 3 cc. concentrated sulphuric acid.

The principle of the Fürth Charnass method<sup>18</sup> is the following:

In a distilling flask with side tube the lactic acid solution, containing 0.5% sulphuric acid (this low concentration will not destroy the lactic acid in heating for 1 to 2 hours) and not more than 0.4 gm. lactic acid, is oxidized with N/10 or N/20 sodium permanganate solution according to the reaction:



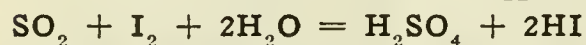
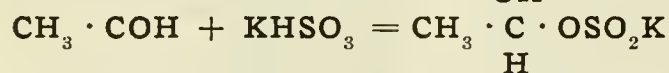
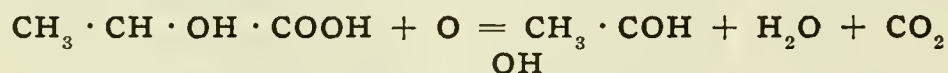
Thus acetaldehyde is formed, which is distilled through a condenser, connected with the side tube of the distilling flask. The other end of the condenser tube is connected with a glass tube of large caliber, the end of which is immersed in a cylinder (500 cc.) containing 50-100 cc. distilled water and enough M/10 or M/20  $\text{KHSO}_3$  solution (6-12 gm.  $\text{KHSO}_3$  per liter) to correspond to at least double the amount of acetaldehyde formed (for 0.1 gm. lactic acid 40 cc. N/10  $\text{KHSO}_3$  solution is used). The acetaldehyde forms a bisulphite compound with potassium bisulphite, which then is determined iodometrically.

The cylinder may also be closed with a stopper containing a water trap to prevent loss of acetaldehyde, which is absorbed in pure distilled water. After the distillation this water or an aliquot part of it, containing the dissolved acetaldehyde, is shaken in a stoppered volumetric flask with double the amount of bisulphite solution and allowed to stand for 15 minutes.

The point of a buret is inserted through a rubber stopper in the neck of the distilling flask. This buret is filled with the permanganate solution (N/10 or N/20). The lactic acid solution (600 cc.) in the distilling flask (1 liter), which contains 0.5% sulphuric acid, is heated to boiling and all air in the apparatus is driven off. Now the sodium bisulphite is added to the water (50 cc.) in the cylinder, and from the buret the permanganate is slowly dropped into the boiling solution, oxidizing the lactic acid. The permanganate drops are so regulated, that the next drop falls when the red color in the boiling solution, produced by the previous drop, has already disappeared. The end point of the oxidation is reached when the solution shows a brown color from a precipitate of manganous superoxide. A few additional cc. of the permanganate are added and the boiling continued until about 100 cc. more is distilled over.

The excess of sodium bisulphite added is determined by titration of the receiver solution with N/10 or N/20 iodine solution, using starch as indicator. The bisulphite solution itself must be standardized with iodine at every determination.

Calculation: If the receiver solution, containing potassium bisulphite, was equal to x cc. N/10 iodine solution before the determination and to y cc. after the determination, the amount of lactic acid in the distilling flask can be calculated from the difference x-y (consumption of N/10 iodine) as follows:



Thus 1 cc. N/10 lactic acid = 2 cc. N/10  $\text{KMnO}_4$  = 1 cc. N/10 acetaldehyde = 2 cc. N/10 iodine solution.

The consumption of N/10 iodine solution (x-y) should then theoretically be equal to the number of cc. of N/10 permanganate used, if only lactic acid is present. If, however, there are oxidizable substances other

than lactic acid in the solution, the amount of permanganate used will be larger than the iodine amount.

If *pyruvic acid* is present, together with lactic acid in the solution to be examined, it may be oxidized by the permanganate to acetic acid and carbon dioxide, and perhaps in part to formic acid. *Lieben*<sup>17</sup> found such an oxidation of pyruvic acid with 2% potassium bichromate.

1 cc. N/10 iodine solution =  $\frac{1}{2}$  cc. N/10 acetaldehyde =  $\frac{1}{2}$  cc. N/10 lactic acid = 0.0045 gm. lactic acid. Each cc. of N/10 iodine, therefore, is equal to 0.0045 gm. lactic acid. However, Fürth has found from a large number of experiments that a loss of 8-10% usually takes place, and he therefore uses the factor 0.005 instead of 0.0045. This factor, 0.005, has been used for the calculations in this investigation. By dividing by the equivalent weight of lactic acid (90) and multiplying by 1000, the lactic acid values will be expressed as cc. 1-N acid.

Urine, treated with ammonium sulphate, was also directly distilled in the Fürth Charnass apparatus without previous shaking with amyllic alcohol to determine lactic acid (Table III, column 2).

### Discussion of the Experimental Results

TABLE III

Lactic acid and pyruvic acid in the 24-hour urine specimen as cc. 1-N acids.

	1	2	3	4	5	
Date 1923	Lactic acid + pyruvic acid; extraction with amylalcohol + reduction with zinc and HCl	Lactic acid; direct determin- ation without extraction and reduction	Lactic acid; ex- traction with amylalcohol but no reduction	Pyruvic acid; 1—2 or 1—3	$\beta$ -Hydroxy- butyric acid.	
Nov. 20 21 22	5.0 13.0 14.5	6.2 5.0 .....	..... 5.7 .....	0 7.3 .....	+ Traces 0	<i>Period II.</i> Natural fat 77 gm. Odd " 100 " CH. 20 Protein 100 "
23 24	..... 7.3	..... 8.1	..... .....	..... 0	+ +	<i>Period III.</i> Natural fat 177 gm. CH. 20 " Protein 100 "
25 26	4.3 4.6	4.7 5.5	..... .....	0 0	0 0	<i>Period IV.</i> Natural fat 177 gm. CH. 70 " Protein 100 "

In Table III, the results obtained are shown. The different acids are here calculated as cc. 1-N acids in the total 24-hour urine specimen.



*Column 1* represents the values obtained by extraction of the urine with amylic alcohol and reduction of the extracts with zinc and hydrochloric acid, to reduce pyruvic acid, if any was present, to lactic acid (see page 163). The figures then show the amount of *lactic acid + pyruvic acid* (as cc. of 1-N acids).

*Column 2* shows the result of *lactic acid* determinations direct on the urines (treated with ammonium sulphate) without previous extraction with amylic alcohol and without reduction with zinc and hydrochloric acid. These values, which represent only *lactic acid*, are a little higher (except on the 21st) than the corresponding figures in column 1, depending upon the presence of some unknown substances (not lactic acid) in the ammonium sulphate treated urines, which on distillation give acetaldehyde and which are not extractable with amylic alcohol.

On November 21, the quantity of lactic acid + pyruvic acid (column 1) was found to be 13.0 cc. of 1-N acids. Column 2 shows the value, 5.0 cc., for the lactic acid. In *column 3*, another value of 5.7 cc. is shown for *lactic acid*; this is obtained by previous extraction with the amylic alcohol but without reduction. The difference, 1 — 2 or 1 — 3, then represents *pyruvic acid* formed (see column 4).

We cannot say exactly what the relative proportion between lactic acid and pyruvic acid was on November 22. A single determination of lactic acid direct in the ammonium sulphate urine seems to show that on this day *considerably more lactic acid than pyruvic acid was formed*.

On the 20th, 24th, 25th and 26th *only lactic acid* appeared to be present in the urine.

We cannot from this single experiment draw an exact conclusion as to whether only lactic acid or only pyruvic acid or both at once were formed from the odd fat.

If both acids are formed, we cannot say whether lactic acid or pyruvic acid is the primary product.

To be concise, we will hereafter use the term, "*Lactic acid + pyruvic acid*," when we mean only lactic acid or only pyruvic acid or both.

In *column 5*, qualitative tests on  $\beta$ -hydroxybutyric acid are given. A comparison between the values in columns 1 and 5 will show that the presence of  $\beta$ -acid does not interfere with the method used for the lactic acid determination.

In figure 2, the results obtained are shown graphically as a function of the experimental time. The curves of the acetone bodies (E), total organic acids (D) and volatile acids (C) from figure 1 are also drawn here.

From curve A ("*lactic acid + pyruvic acid*"), we see that *during the odd fat period II there was an increased formation of "lactic acid + pyruvic acid."* The highest value was reached on the 22nd, the last day of the odd fat period II. By changing the diet from odd to natural (even) fat (protein and carbohydrate

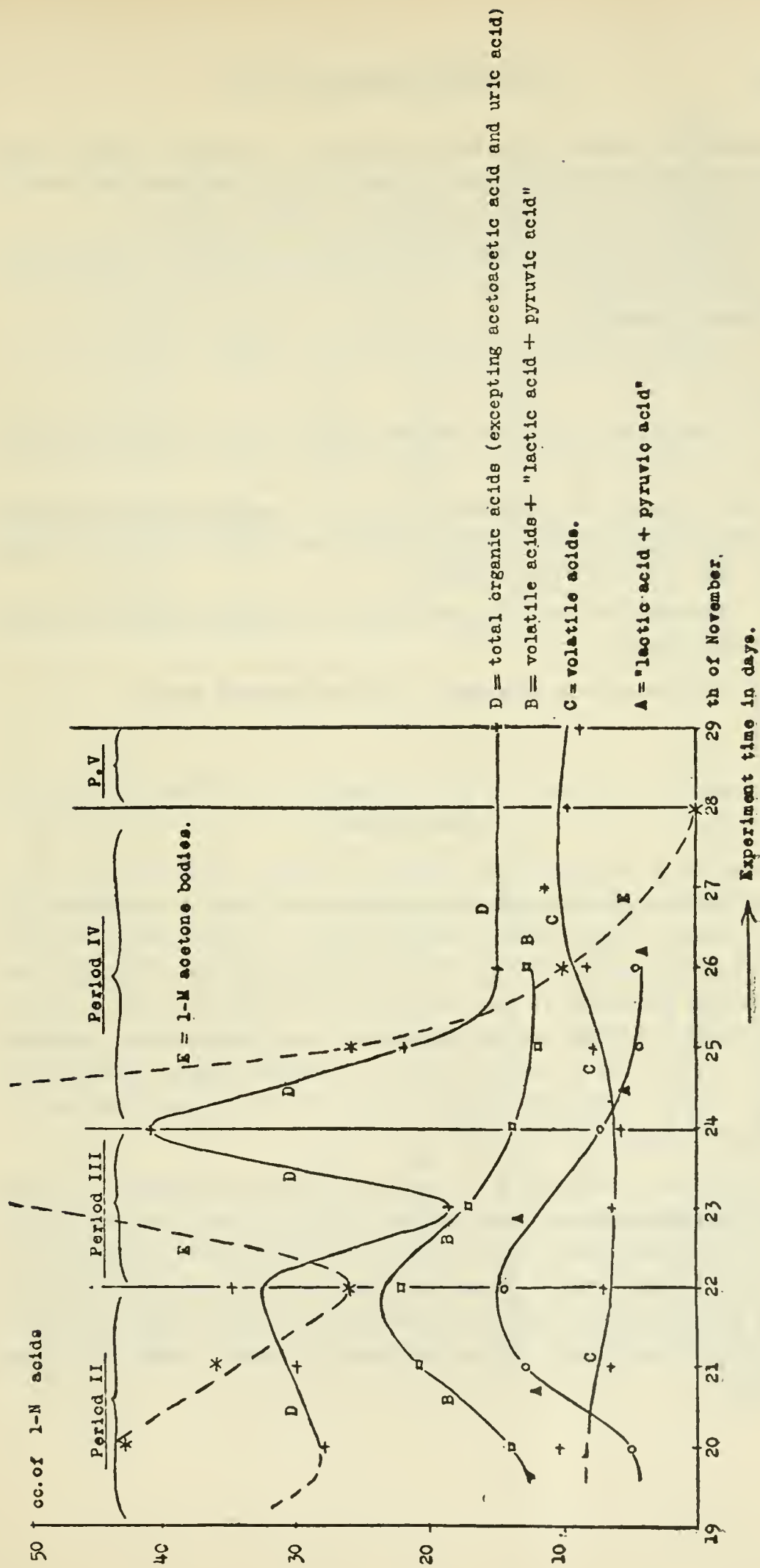


Fig. 2.

unchanged, period III) the "*lactic acid + pyruvic acid*" in less than three days dropped to a normal level. However, an acidosis developed as a result of formation of acetone bodies.

This proves that an abnormal catabolism of odd fat produced in a normal man by a low carbohydrate and high odd fat diet (protein normal):

1. Results in the formation of lactic acid or pyruvic acid or both.
2. Does not form any  $\beta$ -hydroxybutyric acid and acetoacetic acid.

It also proves, on the other hand, that an abnormal catabolism of natural (even) fat, produced by a low carbohydrate and high even fat diet (protein normal):

1. Results in the formation of  $\beta$ -hydroxybutyric acid and acetoacetic acid.
2. Does not form any lactic acid or pyruvic acid.

#### *Theoretical Summary of the Catabolism of Normal Saturated Fatty Acids*

According to *Knoop and Embden*, aliphatic and aromatic saturated fatty acids in their catabolism in the body are oxidized by the  $\beta$ -carbon atom; the  $\alpha$ -carbon atom, together with the carboxyl group, are thereby split off (probably with formation of oxidation products of the type of glyoxylic and formic acids). Thus a loss of two carbon atoms at a time takes place. Normal fatty acids with an *even* number of carbon atoms then finally yield *butyric acid*, and normal fatty acids with an *odd* number of carbon atoms *propionic acid*.

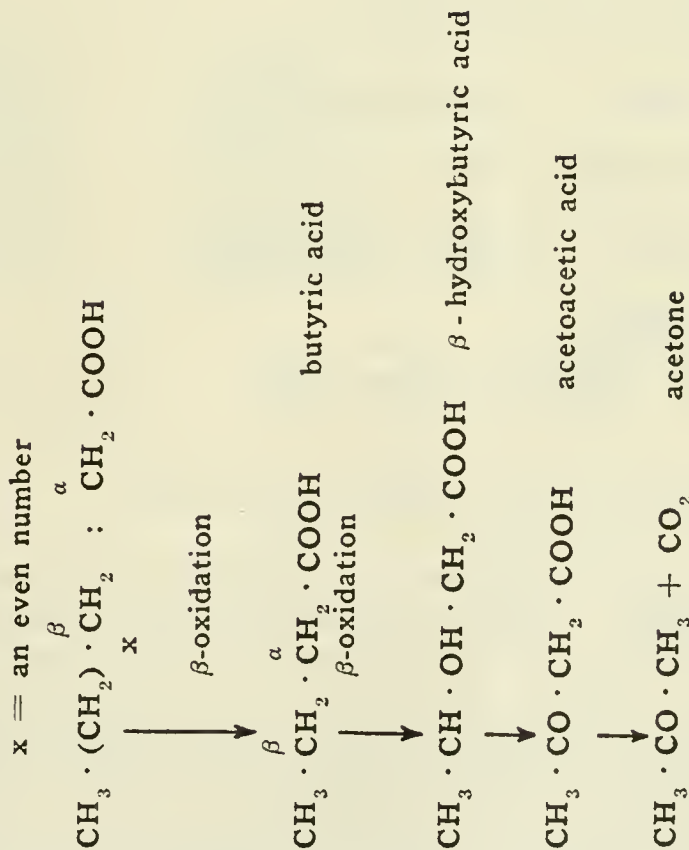
*Even carbon fatty acid fat*, under certain circumstances, forms  *$\beta$ -hydroxybutyric acid and acetoacetic acid* (from *butyric acid*). In this investigation it has been shown that under the same conditions (under which even fatty acids form  *$\beta$ -hydroxybutyric acid and acetoacetic acid* in a normal man), *odd carbon fatty acid fat* forms *lactic acid or pyruvic acid or both* (from *propionic acid*).



The schema represents the probable reactions:

I

*Catabolism of normal saturated fatty acids with an even number of C-atoms.*

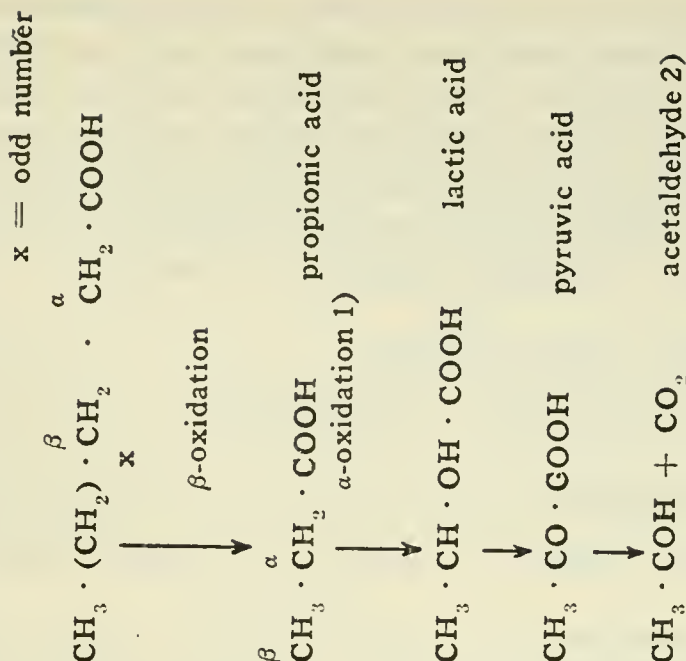


1. Note oxidation of the α-carbon atom. Normal β-oxidation with formation of ketonic acid is no longer possible with a three-carbon acid.

2. The formation of acetaldehyde is not proved, but probable.

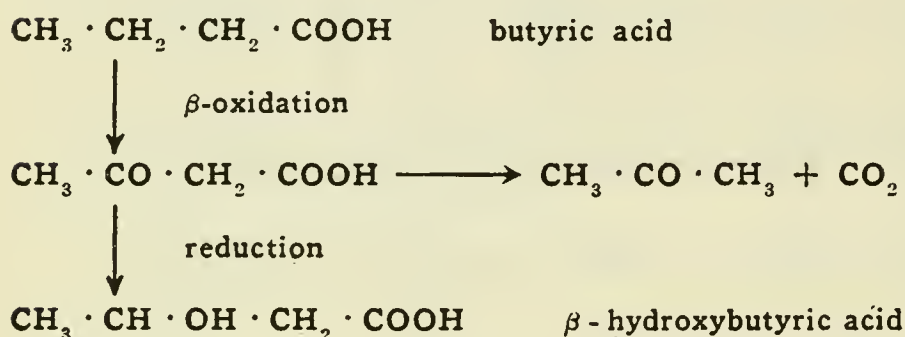
II

*Catabolism of normal saturated fatty acids with an odd number of C-atoms.*

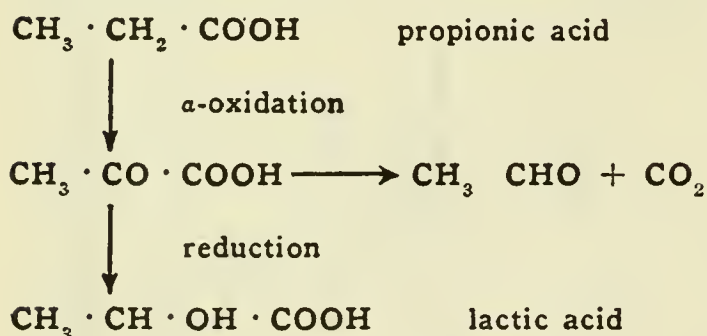


However, it has been found (Blum) that subcutaneous administration of  $\beta$ -hydroxybutyric acid may not be followed by an increased acetoacetic acid formation; on the other hand  $\beta$ -hydroxybutyric acid is formed when acetoacetic acid is introduced. Therefore, it seems that the acetoacetic acid, at least partly may be the original substance from which part of the  $\beta$ -hydroxybutyric acid is formed by a process of reduction.

The schema I then would be:



The analogous schema II would be:



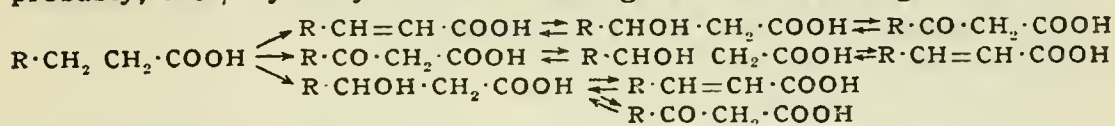
It is probable that both reactions are represented.

Under normal conditions it is believed that the acetoacetic acid is oxidized to acetic acid and carbonic acid in turn and thence to carbon dioxide and water. In the diabetic and also in the normal person, in the absence of carbohydrate in the diet, the oxidation of acetoacetic acid is not complete and acetone bodies appear in the blood and urine.

The only difference between the catabolism of odd fat and the catabolism of even fat, described in the foregoing paragraph,

seems to be that instead of acetoacetic acid and  $\beta$ -hydroxybutyric acid being formed, we have lactic acid and pyruvic acid.\*

The initial products of the oxidation of normal saturated fatty acids may be either the unsaturated acids, the ketonic acids, or, rather less probably, the  $\beta$ -hydroxy acids. Dakin<sup>19</sup> gives the following schema:



Dakin perfused surviving livers, under as nearly similar conditions as possible, with unsaturated ( $\alpha$ - $\beta$ -hexenic acid),  $\beta$ -hydroxy, and  $\beta$ -ketonic (butyrylacetic acid) acids derived from caproic acid, but could not obtain any satisfactory answer as to whether an unsaturated,  $\beta$ -hydroxy, or  $\beta$ -ketonic acid is first formed by the oxidation of caproic acid, and further states that "it would appear more probable that all the acids are in readily shifting equilibrium with each other and are easily interconvertible."

TABLE IV

$\beta$ -hydroxybutyric acid + non-volatile acids, other than lactic acid, pyruvic acid and acetoacetic acid in the 24-hour urine specimen as cc. 1-N acids.

	1	2	3	
Date	Volatile acids + lactic acid + pyruvic acid; = the summary of the corresponding ordinates of curves A and C	$\beta$ -Hydroxybutyric acid + non-volatile acids other than lactic acid, pyruvic acid and acetoacetic acid; = the difference between the corresponding ordinates of curves D and B.	$\beta$ -Hydroxybutyric acid	
20 21 22	14 21 22	14 9 9(?)	+ Traces 0	<i>Period II.</i> Natural fat 77 gm. Odd " 100 " CH. 20 " Protein 100 "
23 24	18 14	1 27	+ +	<i>Period III.</i> Natural fat 177 gm. CH. 20 " Protein 100 "
25 26	12 13	10 2	0 0	<i>Period IV.</i> Natural fat 177 gm. CH. 70 " Protein 100 "

\* "Blum and Woringer have recently shown that when propionic acid is given to normal dogs and rabbits, lactic and pyruvic acids are excreted, and these undoubtedly represent intermediary products of the oxidation of propionic acid, although it is not clear which of these two acids is first formed or whether perchance they are formed from acrylic acid, which Schwenken has shown to be almost quantitatively converted into glucose in the phlorizinized dog. The conversion of propionic acid into lactic acid explains the conversion of the former acid into glucose in the phlorizinized animal, for, as is well known, the conversion of lactic acid into glucose under these conditions is virtually quantitative." Dakin, *Physiol. Rev.*, 1921, 1, 406.



*$\beta$ -hydroxybutyric acid, etc.*

The figures in *column 1* in Table IV are obtained by addition of the corresponding ordinates of the curves of "volatile acids" and "lactic acid + pyruvic acid" in figure 2. Thus they represent the summary of these acids (as cc. 1-N acids in the 24-hour urine). In figure 2, this summary is shown graphically (curve B). As the volatile acids are nearly constant, this curve runs parallel with curve A.

The figures in *column 2*, in Table IV, are obtained by subtraction of the ordinates of curve B (volatile acids + lactic acid + pyruvic acid) from the corresponding ordinates of the total organic acid curve, D, in figure 2. Thus these figures represent the formed amount of  *$\beta$ -hydroxybutyric acid + non-volatile acids other than lactic acid, pyruvic acid and acetoacetic acid* (acetoacetic acid is not contained in the figures for the organic acids) as cc. of 1-N acids in the 24-hour urine. Figure 3 illustrates these acids graphically. (Curve I.) The acetone bodies curve, II, is also shown here.

From curve I (figure 3) we can see that the  *$\beta$ -hydroxybutyric acid*, formed from the *natural fat diet in period I, during the odd fat period II rapidly decreases. During the natural fat period III, the amount of  $\beta$ -acid again increases, and with addition of more carbohydrate to the diet (period IV) the acid soon disappears.*

On the 19th and 20th, there was eliminated a certain amount of  *$\beta$ -hydroxybutyric acid*; on the 21st only traces could be found, and on the 22nd no acid at all (see Table IV). Still the ordinates of the curve (I) are rather high on the 21st and 22nd (period II).

This seems to show that odd fat can form small amounts of some non-volatile acids other than lactic acid and pyruvic acid (not  *$\beta$ -hydroxybutyric acid* and acetoacetic acid). The rapid rise and fall of curve I, between the 23rd and 26th, was probably due only to  *$\beta$ -hydroxybutyric acid*, which was formed during the natural fat period III and disappeared on addition of more carbohydrate to the diet during period IV.

However, it is probable that part of the free lactic acid and pyruvic acid, formed during the odd fat period II, was converted into a lactid compound as mentioned on page 162. It is then possible that this lactid compound (by the treatment with ammonium sulphate) at a slight alkaline reaction (see page 163) was not completely converted into the normal acid salt. As a lactid compound, in the determination of "lactic acid + pyruvic acid," is stable in amylic alcohol upon further treatment with carbonate solution, we surmise that the amount of "lactic acid + pyruvic acid" is somewhat diminished. Thus the figures in Table IV, column 2, on the 20th, 21st and 22nd, may be too high and curve I may run too high over the X-axis.

Further investigations are necessary to decide if odd fat can form non-volatile acids (which are not  *$\beta$ -hydroxybutyric acid* or

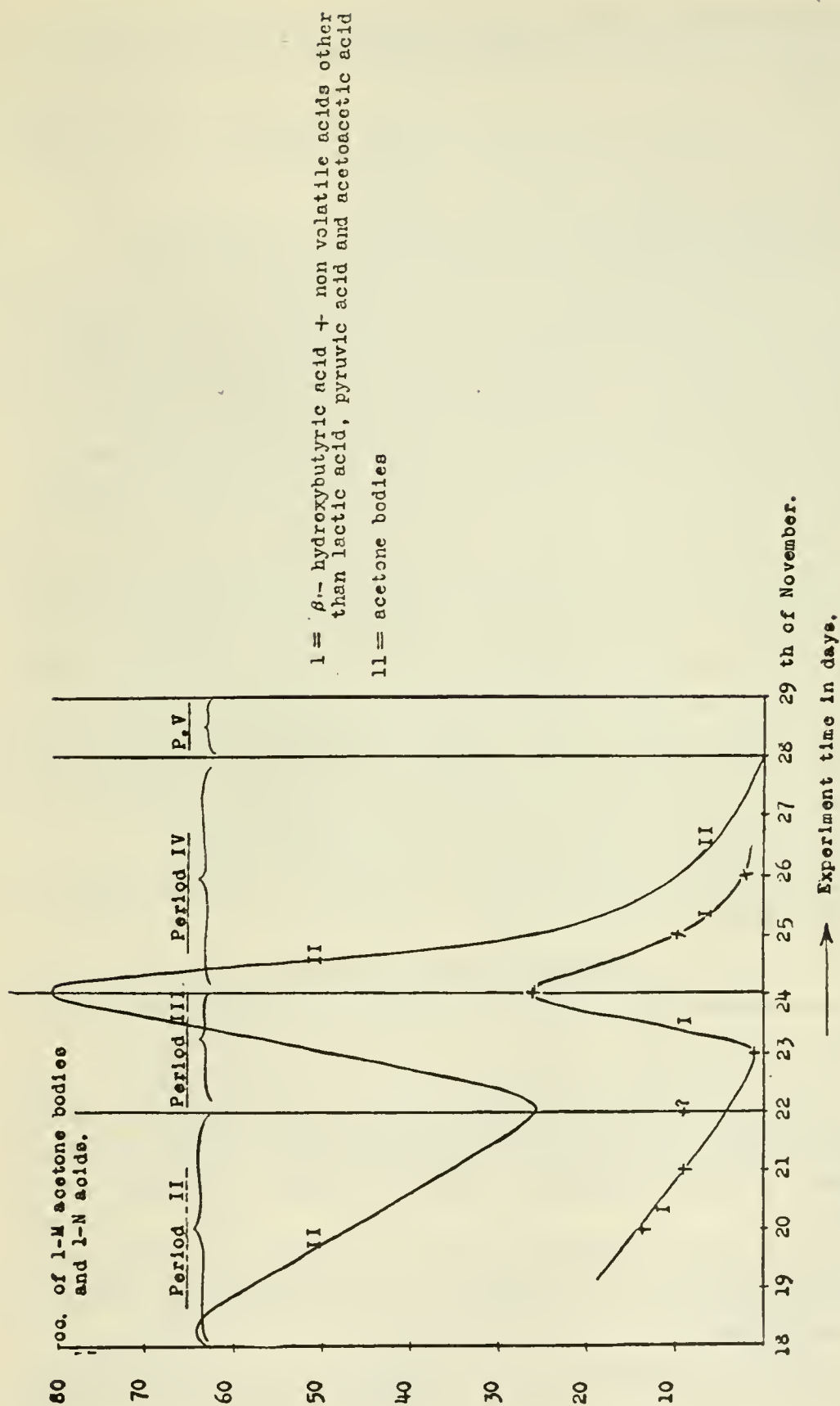


Fig. 3a.

acetoacetic acid), other than lactic acid and pyruvic acid; for example, the unsaturated acid derived from propionic acid (acrylic acid).

This investigation has given another *proof* for the  *$\beta$ -oxidation theory*.

Of clinical interest is the fact that odd fat develops acidosis in a normal man under the same conditions as natural fat.

An acidosis produced by a high natural fat and low carbohydrate diet in a *normal man does not disappear upon replacing the natural fat by odd carbon fat* (though the acetone bodies decrease); instead of *the acidosis caused by natural fat* from the *oxidation products of butyric acid* ( $\beta$ -acid, acetoacetic acid), another *acidosis is formed from the odd fat by the oxidation products of propionic acid* (lactic acid, pyruvic acid, acrylic acid?).

The blood sugar content of the normal man was not influenced by the odd fat (see Table I). The "lactic and pyruvic" acids formed were not or at least not completely converted into glucose in the body, but were partly or wholly excreted through the kidneys. In a non-diabetic patient (case 1526) in Modern's paper (page 185) we find the statements in this investigation confirmed. The feeding of odd carbon fatty acid fat here resulted in an acidosis (low carbon dioxide combining power of the blood) from a formation of "lactic acid and pyruvic acid" in the blood and only a small increase of the blood sugar.

In a diabetic, the conversion of lactic acid into sugar may possibly require consideration.

### *Summary and Conclusions*

These observations can be summed up in the following conclusions:

1. In a normal man with absence or insufficiency of carbohydrate in the diet, acidosis is produced by a metabolic breakdown of natural fats (which are all glycerides of fatty acids containing an even number of carbon atoms), yielding acetone bodies.

2. Odd carbon fatty acid fat in its catabolism in the human body with absence or insufficiency of carbohydrate forms no acetone bodies.

3. Ketosis produced in a normal man by a combined high natural fat (177 gm.) and low carbohydrate (20 gm.) diet (with



normal protein) can be cleared up in a few days by replacing 100 gm. of the natural fat with 100 gm. of odd carbon atom fatty acid fat, or by increasing the quantity of carbohydrate to 70 gm. per day (fat diet unchanged).

4. With small amounts of acetone bodies in the urine,  $\beta$ -hydroxybutyric acid could not be isolated among the excreted acetoacetic acid and acetone. It was only when the acetone bodies increased that  $\beta$ -hydroxybutyric acid appeared.

5. The volatile acids in the urine seem under the same conditions to undergo no important qualitative or quantitative change with either odd fat or natural fat diet. The main part of the volatile acids seems to consist of formic acid. Acetic acid and propionic acid are not found (with either odd fat or natural fat diet) or occur only in very small amounts among the volatile acids.

6. By increasing the carbohydrate from 20 to 70 gm. per day with the same high (177 gm.) fat diet (and normal protein), there was a slight increase in the amount of volatile acids.

7. According to Knoop and Embden, aliphatic and aromatic saturated fatty acids in their catabolism in the body split off two carbon atoms at a time. Normal fatty acids with an even number of carbon atoms thus finally yield butyric acid; and normal fatty acids with an odd number of carbon atoms yield propionic acid.

An abnormal catabolism of even carbon atom fatty acid fat, produced by a low carbohydrate and high even fat diet (protein normal); (a) results in the formation of  $\beta$ -hydroxybutyric acid and acetoacetic acid, through a  $\beta$ -oxidation of the butyric acid; (b) does not form any lactic acid or pyruvic acid.

An abnormal catabolism of odd carbon fatty acid fat, produced in a normal man by a low carbohydrate and high odd fat diet (protein normal); (a) results in the formation of lactic acid or pyruvic acid or both, through an  $\alpha$ -oxidation of the propionic acid; (b) does not form any  $\beta$ -hydroxybutyric acid or acetoacetic acid.

8. Further investigations are necessary to decide if odd fat can form non-volatile acids (which are not  $\beta$ -hydroxybutyric acid and acetoacetic acid), other than lactic acid and pyruvic acid.

9. The results obtained furnish another proof for the  $\beta$ -oxidation theory.

10. Odd carbon atom fatty acid fat, like the even carbon variety, requires carbohydrate for its normal or complete utilization.

11. An acidosis produced by a high natural fat and low carbohydrate diet in a *normal man* does not disappear upon replacing the natural fat by odd carbon fat (though the acetone bodies decrease); instead of the acidosis caused by natural fat from the oxidation products of butyric acid ( $\beta$ -acid, acetoacetic acid) another acidosis is formed from the odd fat by the oxidation products of propionic acid (lactic acid, pyruvic acid, acrylic acid?)

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# CLINICAL OBSERVATIONS WITH ODD-CARBON-ATOM FAT (INTARVIN).

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The use of an odd-carbon fat in diabetic diets was recommended by Kahn,<sup>1</sup> who first succeeded in preparing it in quantity. Clinical trials were made in this Institute with this new fat, which was kindly furnished for the purpose by the Intarvin Company, 80-86 Hancock Street, Long Island, N. Y.

## CLINICAL OBSERVATIONS.

We did not have culinary success equal to that described by Kahn. We found that the odd-carbon fat when used for frying of foods became solid as soon as removed from the stove. It did not mix with natural fat. No flavoring agent was found to cover its smell and taste, unless used in quantities which were inedible. We tried also to remove the low fatty acids to which we attributed the characteristic smell and taste. Shaking with water up to half an hour failed to do so. Emulsification by means of 0.5 per cent.  $\text{Na}_2\text{CO}_3$  solution or accurate neutralization with normal N/10 NaOH also had no important effect. We obtained the best result by emulsification and reprecipitation with N/10 HCl solution nearly to the neutral point, when a semi-solid paste was formed, which was practically tasteless and odorless and better suited for cooking. This method, however, proved too tedious to be carried out on a sufficiently large scale for our experiments. We finally found that mineral oil served better than anything else to keep the odd-carbon fat in liquid form, and this method was sometimes used in serving. It was distributed between the three regular meals of the day, but perhaps because the patient swallowed it in large doses like medicine we found a comparatively high excretion of the artificial fat in the feces. Patient No. 54 excreted 3 to 5 per cent. of natural fat, while the figures for the odd-carbon fat ranged as high as 15 to 25 per cent.

Utilization: The part which was absorbed seemed to be utilized like natural fat. It was counted in our experiments as having the same food value as natural fat. Storage in the body was indicated by increase in weight. Patient No. 1172 (table 2) gained four pounds during the odd fat feeding, a larger gain than in any previous or subsequent period on the same caloric intake.

Nitrogen Metabolism: It is fairly plain from the record of patient No. 54 (table 1) that the artificial fat spared nitrogen and favored nitrogen storage. When undernutrition was instituted November 1, by omitting 150 grams of odd-carbon fat, the nitrogen balance became negative.



**Subjective Effects:** Nausea was present in every case. Vomiting occurred in four out of seven. Gastrointestinal distress followed in three cases. Marked constipation was present in every case. In addition, every patient complained of marked muscular weakness, together with dull sensations in the head which occasionally increased to real headaches. These difficulties prevented carrying out the tests in such complete form as would have been desirable.

### CASE REPORTS.

I. Patient No. 54 was a woman with very severe diabetes, who has been described in previous publications from this Institute.<sup>2</sup> She had lived in the Institute continuously for 4 years, and on undernutrition diets of about 1000 calories she had been kept with normal blood sugar in extreme emaciation at weights as low as 69 pounds. Beginning in August, 1922, with increasing doses of insulin she had been raised to the level of diet and weight shown in table 1.

#### *Remarks.*

*Diet.*—The regime was one of overnutrition, as shown by the increase of weight from 112 to 117 pounds. The diet was low in carbohydrate and high in fat, to favor the occurrence of acidosis. September 30, the protein was reduced to 80 gm. and the carbohydrate to 15 gm., in the attempt to increase acidosis.

September 16 to 19, 100 gm. of natural fat was replaced by 100 gm. of intarvin, keeping the total calories supposedly unchanged. September 20, the use of natural fat alone was resumed.

In two subsequent periods (Oct. 4-10, Oct. 22-31), comparisons were made by substituting 150 gm. of natural fat in the diet by 150 gm. of intarvin. In the two final periods shown in the table, comparison was made with undernutrition by merely omitting 150 gm. of intarvin from the diet and not replacing it with any other food.

*Ketosis.*—In the blood plasma, the nitroprusside reaction was never more than faint and no significant quantities of acetone bodies were found. The ketonuria also remained slight, illustrating the long-familiar ability of many patients to remain free from dangerously high ketosis on diets largely overbalanced with fat, so long as they are nearly or completely free from glycosuria. The ketogenesis with intarvin was plainly less than with natural fat in the diet, as shown particularly in the period Oct. 22-31. On the other hand, intarvin did not act as if it were equivalent to carbohydrate, because the last traces of ketonuria were not cleared up.

Furthermore, in the undernutrition period, Nov. 1-25, the ketosis was just as slight as in any intarvin period, though the fall of weight showed that body fat was being burned in addition to the fat of the diet. The higher ketosis in the final undernutrition period, Nov. 30-Dec. 19, is explained by the existing glycosuria due to reduction of insulin.

*Acidosis.*—With three exceptions, the plasma bicarbonate values may be considered as slightly subnormal, and they were not raised to normal by intarvin (though at a later period, not shown, they were raised to normal by increase of carbohydrate). The urinary ammonia averaged distinctly higher in the intarvin period, Oct. 22-31, than in the preceding natural-fat period (Oct. 11-21) or the ensuing undernutrition period (Nov. 1-25). The higher ammonia in the final undernutrition period in December merely accompanied the glycosuria and ketonuria at that time. The urinary acidity can be compared only for the brief intarvin period, Nov. 26-29, but it was not reduced during this time. These facts can be explained in the light of Lundin's studies, by the assumption that the odd-carbon fat gives rise, not to acetone bodies, but to other acids such as lactic and pyruvic, and further that the odd-carbon fat requires carbohydrate for its complete combustion as truly as does natural fat.

*Tolerance and insulin requirement.*—If any large proportion of the intarvin had been directly converted into sugar, marked glycosuria must have resulted from the substitution of intarvin for natural fat, since Sherrill<sup>3</sup> in particular has shown that the immediate glycosuric influence of carbohydrate is much stronger than that of an isocaloric quantity of fat. On the other hand there is no evidence that intarvin imposes a less burden on the tolerance than natural fat. Before the first intarvin period (Sept. 16-19) the plasma sugar was already falling, and it remained at 0.162-0.166 per cent. both with intarvin and with natural fat. In the next intarvin period (Oct. 4-10) a reduction of insulin from 60 to 50 units per day resulted in a prompt rise of plasma sugar. There was then no appreciable change on returning to natural fat (Oct. 11). After the reduction of insulin to 42 units (Oct. 16) there was a further rise of plasma sugar which culminated in slight glycosuria in the intarvin period beginning Oct. 22. Omission of the intarvin (Nov. 1), producing undernutrition led to a quick cessation of glycosuria and fall of plasma sugar.







TABLE 1—Continued

Date, 1923	DIET				URINE							BLOOD				Insulin Units	Body Wgt., lbs.	Remarks		
	P Gm.	Fat Gm.	CH Gm.	Odd Carbon Fat Gm.	Total Cal- ories	Vol. cc.	Dext. Gm.	Qual. Nitro- Pruss- ide	Total Ace- tone Bodies Gm.	T.N. Gm.	NH <sup>3</sup> Gm.	Total Acid (C.C. of n-1) NaOH	Plasma Sugar Mg. per 100 cc.	Plasma NaCl Mg. per 100 cc.	Plasma Nitro- Pruss- ide Test				CO <sub>2</sub> Vol. per Ct.	
Oct. 26	....	....	....	....	....	1675	vft	vft	0.44	11.9	0.97	....	....	....	....	....	....	42	124	
Oct. 27	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	42	125	
Oct. 28	....	....	....	....	....	1600	vft	vft	tr	12.3	0.7	....	....	....	....	....	....	....	....	
Oct. 29	....	....	....	....	....	2000	0	vft	tr	7.5	1.1	....	....	....	....	....	....	42	124	
Oct. 30	....	....	....	....	....	1200	vft	vft	tr	6.4	0.97	....	....	....	....	....	....	42	124	
Oct. 31	....	....	....	....	....	1800	vft	vft	tr	11.2	0.7	....	....	....	....	....	....	42	....	
Nov. 1	80	89	15	0	1181	1300	vft	vft	tr	13.1	0.96	....	272	573	vft	49.8	42	124		
Nov. 2	....	....	....	....	....	1900	0	vft	tr	11.8	0.8	....	....	....	....	....	....	42	....	
Nov. 3	....	....	....	....	....	1800	0	vft	tr	12.0	0.8	38.8	....	....	....	....	....	42	126	
Nov. 4	....	....	....	....	....	1800	0	vft	tr	11.9	0.74	36	150	548	0	46.6	42	....	....	
Nov. 5	....	....	....	....	....	1600	0	ft	tr	12.2	0.77	42.6	....	....	....	....	....	42	124	
Nov. 6	....	....	....	....	....	2000	0	vft	tr	13.4	0.8	27.2	....	....	....	....	....	42	....	
Nov. 7	....	....	....	....	....	1400	0	ft	0.3	14.3	1.0	47	206	581	vft	57.6	42	122		
Nov. 8	....	....	....	....	....	1600	0	vft	tr	13.4	0.8	38.5	....	....	....	....	....	42	....	
Nov. 9	....	....	....	....	....	1700	0	vft	tr	13.8	0.7	31.3	....	....	....	....	....	42	122	
Nov. 10	....	....	....	....	....	1600	0	ft	tr	13.2	0.9	35.2	....	....	....	....	....	42	....	
Nov. 11	....	....	....	....	....	1600	0	vft	tr	12.0	0.8	32.0	....	....	....	....	....	42	122	
Nov. 12	....	....	....	....	....	1500	0	vft	tr	11.9	0.6	33.6	166	536	vft	44.3	42	....	....	
Nov. 13	....	....	....	....	....	1800	0	vft	tr	12.9	0.7	....	....	....	....	....	....	42	123	
Nov. 14	....	....	....	....	....	1600	0	vft	tr	14.7	0.8	39.0	....	....	....	....	....	42	....	
Nov. 15	....	....	....	....	....	1900	0	vft	tr	13.6	0.7	31.2	200	552	vft	45.2	28	123		
Nov. 16	....	....	....	....	....	1300	vft	ft	tr	13.0	0.7	36.0	....	....	....	....	....	28	122	
Nov. 17	....	....	....	....	....	1600	0	ft	tr	13.0	0.7	36.0	....	....	....	....	....	28	122	
Nov. 18	....	....	....	....	....	1400	0	vft	tr	12.0	0.7	36.0	....	....	....	....	....	28	....	





Nov. 15, the insulin was reduced to 28 units, and the sugar in blood and urine was scarcely higher than in the preceding intarvin period with 42 units. Nov. 26-29, the addition of 150 gm. of intarvin resulted in a rapid rise of glycosuria to 27 gm. The ensuing undernutrition period reduced this glycosuria but could not abolish it, as the tolerance had apparently been damaged.

It is unfortunate that the periods of observation were shortened by the nauseating effect of intarvin, because the influence of foods which are not directly convertible into sugar can be studied accurately only with sufficiently long periods. Previous studies<sup>4</sup> from this Institute, however, have proved that the diabetic tolerance and insulin requirement are governed not merely by carbohydrate but by the total caloric intake and the body weight. This rule applied not only to fat but also to alcohol, which is as free from conversion into sugar or acetone as is supposedly intarvin. The supposition that additional calories can be given or the body weight built up by intarvin without imposing an additional burden upon the tolerance or the insulin requirement therefore rests upon a false conception of diabetes. The above demonstration that odd-carbon fat conforms to the same rule as other energy carriers strengthens the proof of this law of total calories in diabetes.

II. Patient No. 1172, female, aged 27 years, has also been described in a previous publication.<sup>5</sup> Three months of insulin treatment had increased her weight by twenty pounds prior to the beginning of this experiment. The findings are shown in Table 2.

### *Remarks.*

*Diet.*—This was an overnutrition regime of 2,800 calories, with an adequate carbohydrate ration of 100 gm. which prevented all ketosis throughout. July 2-7, 100 gm. of natural fat was replaced by 100 gm. of intarvin, and comparison was made with the preceding and following periods without intarvin.

*Tolerance and insulin requirement.*—The existing hyperglycemia showed a general increase in parallel with the increase of body weight. The essential point of the experiment is that the tolerance was not appreciably altered one way or the other by intarvin in comparison with natural fat.

TABLE 2

Case No. 1172

Date 1923	DIET					URINE		Plasma Sugar Mg. per 100 cc.	Insulin (units)	Body Weight (lb.)
	P. Gm.	Fat Gm.	C. H. Gm.	Odd Carbon Fat Gm.	Cals.	Dext. Gm.	Nitro- prus- side			
June										
24	100	222	100	0	2800	0	0	...	21	..
25	...	...	...	...	...	0	0	...	21	96
26	...	...	...	...	...	0	0	...	21	..
27	...	...	...	...	...	0	0	...	21	94
28	...	...	...	...	...	0	0	258	21	..
29	...	...	...	...	...	0	0	...	21	94
30	...	...	...	...	...	0	0	...	21	..
July										
1	...	...	...	...	...	0	0	272	21	94
2	100	122	100	100	2800	0	0	...	21	..
3	...	...	...	...	...	vft.	0	...	21	95
4	...	...	...	...	...	0	0	...	21	..
5	...	...	...	...	...	0	0	283	21	96
6	...	...	...	...	...	0	0	...	21	..
7	...	...	...	...	...	0	0	...	21	..
8	100	222	100	0	2800	0	0	230	21	..
9	...	...	...	...	...	00	0	...	21	98
10	...	...	...	...	...	vft.	0	...	21	..
11	...	...	...	...	...	vft.	0	...	21	..
12	...	...	...	...	...	0	0	...	21	..
13	...	...	...	...	...	0	0	239	21	98
14	...	...	...	...	...	0	0	...	21	..
15	...	...	...	...	...	0	0	...	21	..
16	...	...	...	...	...	vft.	0	300	21	98
17	...	...	...	...	...	0	0	...	21	..
18	...	...	...	...	...	0	0	...	21	99

III. Patient No. 1526, male, Hebrew, aged 47 years, had hypertension for the last four years. The onset was acute with dizziness, headaches and shortness of breath. The condition was rapidly progressive and various forms of treatment failed to relieve subjectively or symptomatically. On admission to the Institute on July 17, 1923, the blood pressure was 208-120. The physical examination, apart from marked atherosclerosis, dilatation and hypertrophy of the heart and aorta, was essentially negative. There was no evidence of diabetes.

TABLE 3  
Table No. 1526

Date	DIET				URINE			BLOOD PLASMA				Insulin (units)	Body Weight (lb.)
	Prot. Gm.	Fat Gm.	CH. Gm.	Odd Carbon Fat Gm.	Total Cals.	Dext. Gm.	Nitro- prusside	T. N. Gm.	Sugar Mg. per 100 cc.	NaCl Mg. per 100 cc.	Nitro- prusside	Plasma CO <sub>2</sub> Cap. Vol. %	
Aug. 26	40	144	12	0	1400	0	ft.	10.3	...	...	...	...	135
27	40	221	18	0	2190	0	ft.	...	...	...	...	...	0
28	40	137	10	0	1435	0	heavy	8.7	...	...	...	...	0
29	40	81	10	0	898	0	heavy	6.0	...	...	...	...	134
30	40	3	0	220	2167	0	ft.	5.2	...	...	...	...	0
31	40	1	0	166	1663	0	mod.	6.3	111	502	heavy	44.3	132
Sept. 1	40	2	0	88	889	0	mod.	7.1	127	536	ft.	32.8	0
2	unweighed diet			0	....	0	ft.	11.2	...	...	...	...	0
3	unweighed diet			0	....	0	0	6.7	135	494	0	...	...



### *Remarks.*

*Diet.*—August 23-29, a state of carbohydrate privation acidosis was induced by means of a diet of 40 gm. protein, 178 to 220 gm. fat, and 10 gm. carbohydrate. On Aug. 26 a faint nitroprusside reaction was present in the urine and increased to heavy by Aug. 28. Clinically the acidosis was evidenced by loss of appetite, nausea, malaise and headaches. Beginning Aug. 30, natural fat was withdrawn from the diet and he was urged to eat as much intarvin as possible. He was allowed the same 40 gm. protein per day and 600 gm. of thrice cooked vegetables as a vehicle to aid in making the odd-carbon fat as palatable as possible. The quantities shown in Table 3 were the utmost he could endure.

*Plasma sugar.*—This rose slightly, but probably only within the limits of accidental variation.

*Ketosis.*—The nitroprusside reactions, which had become heavy in urine and blood plasma on natural fat, diminished to faint traces on the three days of intarvin feeding. In this non-diabetic patient, therefore, the odd-carbon fat was obviously non-ketogenic and possibly anti-ketogenic.

*Acidosis.*—The subjective symptoms which accompanied the ketosis were not relieved by the intarvin. The plasma bicarbonate, which had fallen to 44.3 volues per cent. with the natural fat, with intarvin fell further to 32.8 volumes per cent. It is suggested that an acidosis due to carbohydrate deficiency was still present, but the incomplete combustion of intarvin gave rise not to acetone bodies but to other acids (lactic and pyruvic).

### *Conclusions.*

1. An odd-carbon fat (intarvin) apparently requires carbohydrate for its complete utilization, similarly to natural fat.
2. The incomplete combustion of odd-carbon fat can give rise to an acidosis, due not to acetone bodies but to other acids (probably lactic and pyruvic).
3. The odd-carbon fat creates essentially the same insulin requirement as natural fat. Apparently, therefore, it is not directly converted to any large extent into carbohydrate. On the other

hand any supposition that it can be used without imposing a burden upon the diabetic tolerance is erroneous.

4. The law of the relation of total calories and body weight to the pancreatic island function, previously established for natural fat and alcohol, is further corroborated by the experience with odd-carbon fat.

5. For the above reasons an odd-carbon fat is of no possible value in practical diabetic treatment. On account of the interesting theoretical and experimental opportunities opened up, however, it is a valuable contribution to the subject of diabetes and metabolism.

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## EXPERIMENTAL STUDIES IN DIABETES

### SERIES V.—ACIDOSIS

#### 3. *Acidosis in Dogs Without Glycosuria*

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The previous paper<sup>1</sup> has shown that normal dogs ordinarily develop no severe ketosis on fasting or high fat feeding, and on too long continuance of diets overbalanced with fat they develop either cachexia (with indigestion) or a peculiar form of intoxication, without any tendency to coma.

More than fifty dogs were subjected to fasting and feeding tests under different conditions in the attempt to find some way of producing an appreciable acidosis, but as the general results were so negative, only a few records illustrating certain special features will be summarized here.

Dog C3-93, a black male mongrel, aged 2 or 3 years, had been in stock for several months on bread and soup diet, and weighed 16 kg. in a state of medium nutrition.

May 23, 1916, he was suddenly placed on complete fasting, and up to May 31 had developed no acetone reactions in urine or blood plasma.

He was then placed on a diet of 200 gm. lung and 200 gm. suet, which he ate remarkably well, and by July 11 had reached a maximum weight of 16.7 kg., without acetonuria. As the appetite began to fail, the experiment was interrupted and a diet of bread and soup given to prevent cachexia or intoxication.

TABLE 1

Dog C3-93

Hour	Sugar mg. per 100 cc.	CO <sub>2</sub> Capacity Vol. %	Blood Plasma Nitroprusside Reaction	Total Acetone mg. per 100 cc.	Lipemia (qualitative)
A. M.					
8:30	100	44.3	Neg.	21	0
10:30	133	50.0	Neg.	---	+
P. M.					
12:30	100	48.0	Neg.	---	++
2:30	120	-----	Faint	---	+++
4:30	91	46.2	Faint	37	+++



September 20, this diet was changed abruptly to nothing but 300 gm. suet daily. September 27, the dog still ate the suet voluntarily with apparently good digestion, and blood analyses were made at two-hour intervals after feeding at 8:30 A. M.

Total urine for period 140 cc., containing 17 mg. total acetone.

As the appetite failed and diarrhea appeared, the dog was fasted for 3 days, September 29 to October 1, still without significant acidosis.

Beginning October 2, a diet of 150 gm. beef-lung and 250 gm. suet was fed, with talcum powder to control diarrhea. Everything was eaten voluntarily until the end of November, the appetite and digestion of fat being highly exceptional. In December, forcing of food became necessary. The dog met accidental death December 11, when the weight was 14 kg. Eczema or mange had been present and growing worse for the last two months, but there were no other toxic symptoms. Acetonuria was absent or trivial by qualitative and quantitative tests throughout.

Von Noorden<sup>2</sup> and Mohr<sup>3</sup> stated that dogs kept for considerable periods on carbohydrate diet and then subjected suddenly to fasting develop acidosis like human beings. They give no experimental support for the assertion, which is contradicted by this and other experiments in this paper and paper No. 2.<sup>1</sup>

Dog D4-08 was an obese yellow male mongrel, almost toothless with age, but nevertheless vigorous and sexually active. The weight was 25.2 kg. June 30, 1916, the splenic process and most of the body of the pancreas, weighing 30 gm., were removed, leaving the uncinat process and a small part of the body about the main duct. Glycosuria remained absent as anticipated, and the dog thrived on bread and soup diet.

October 3, the diet was changed to 250 gm. beef-lung and 300 gm. suet, which was eaten eagerly. October 5, the dog had a number of violent general convulsions, and in the intervals lay on his side with general twitching of muscles. He was conscious but refused all food. The condition was supposed to be probably rabies, but the dog drank water thirstily without great difficulty and, therefore, was kept under observation. On October 6, he took milk and bread and seemed much better. On September 7 and 8, he appeared well and ate the diet of 250 gm. lung and 300 gm. suet. On October 9, he was well and ravenously hungry, and in addition to the 250 gm. of lung ate over 500 gm. of suet. During the following hours a series of violent convulsions occurred, and between the attacks the dog was delirious and unable to stand. By evening he was merely weak, but otherwise recovered.

October 10 to 17, the health and spirits were excellent on a diet of bread and soup.

October 17, the diet was changed to 200 gm. lung and 300 gm. suet, which was eaten from that time onward without any disturbance of health. This was one of the dogs having an exceptional appetite for fat, and no forcible feeding was necessary until January, 1917. The feces became

TABLE 2  
Dog C3-86

Date 1916	Wgt. kg.	Urine				Blood Plasma			Diet
		Vol. cc.	Sugar gm.	Total N gm.	Total Acetone mg.	Sugar mg. per 100 cc.	CO <sub>2</sub> Cap. Vol. %	Total Acetone mg. per 100 cc.	
Oct. 9	12.8	110	Neg.	3.26	52	---	53.8	18	350 gm. lung and 250 gm. suet.
10	13.0	776	Neg.	8.85	30	---	---	---	350 gm. lung and 250 gm. suet and 10 gm. sodium bicarbonate.
11	---	300	Neg.	5.58	48	---	---	---	350 gm. lung and 250 gm. suet and 10 gm. sodium bicarbonate.
12	---	870	Neg.	8.95	87	---	---	---	350 gm. lung and 250 gm. suet and 10 gm. sodium bicarbonate.
13	---	416	Faint	5.58	46	118	67.2	25	350 gm. lung and 250 gm. suet and 5 gm. sodium bicarbonate.
14	---	498	Neg.	7.35	49	---	---	---	350 gm. lung and 250 gm. suet and 10 gm. sodium bicarbonate.
15	---	171	Neg.	5.38	48	---	---	---	350 gm. lung and 250 gm. suet.
16	---	536	Neg.	8.63	102	116	54.8	21	350 gm. lung and 250 gm. suet.
17	13.8	435	Neg.	7.57	100	164	52.8	---	350 gm. lung and 250 gm. suet.
18	13.9	342	4.13	8.21	72	137	57.6	---	385 gm. lung.
19	---	312	2.45	8.61	78	---	---	---	385 gm. lung.
20	---	320	Faint	8.29	58	---	---	---	385 gm. lung.
21	---	268	Faint	9.00	15	---	---	---	385 gm. lung.
22	---	256	Neg.	8.50	Trace	---	---	---	385 gm. lung.
23	---	226	Faint	7.62	Trace	151	62.4	12	385 gm. lung.
24	13.4	376	2.05	9.40	45	---	---	---	385 gm. lung.
25	---	260	1.95	5.98	Trace	---	---	---	Not fed.
26	---	128	Faint	3.60	0	222	---	0	Not fed.
27	12.8	96	Faint	1.68	0	---	---	---	Not fed.
28	---	120	Faint	3.16	0	100	---	0	Not fed.

more and more fatty, and strength was lost to a degree which was disproportionate to the loss of weight.

March 6, the dog choked to death, largely because of weakness. The weight was still 18.75 kg. The autopsy was negative. The muscles were in excellent condition, and the body still contained more fat than that of a normal dog. The liver, kidneys, adrenals and pancreas were normal both grossly and microscopically.

Out of the entire long series of animals, convulsive attacks coming on immediately during digestion of an excessive fat ration were observed only in one puppy in addition to this senile dog. Their nature, whether a rare toxic condition or a mere symptom of indigestion, is unknown.

Acetone was absent or limited to doubtful traces at all times. The experiment is one of a series showing that obese dogs are not specially disposed to acidosis. The partial pancreatectomy, falling far short of the production of diabetes, was without influence, as was true also in other dogs. Incidentally, the preliminary feeding with carbohydrate created no susceptibility to acidosis.

Dog C3-86 was a potentially diabetic animal possessing only 1-12 of the pancreas. The greater part of the long experimental record has been published in another connection.<sup>4, 5</sup> Acetone reactions were negative except at the period of highest weight, when the urine gave a slight color with nitroprusside. Quantitative determinations were made at this stage as shown in table 2.

This dog had long been on protein or protein-fat diets, which failed to produce any "immunity" against acidosis.

There is probably an influence of the latent diabetes. The previously published record<sup>6</sup> of dog No. 356 gives an illustration of slightly higher ketonuria in a condition of potentially severe diabetes, even when glycosuria was absent and the plasma sugar normal. Other observations have strengthened the impression that a dog with severe latent diabetes is more subject to ketonuria with fasting or fat-rich diet than a normal dog. Precise proof of this impression would be difficult, because the acetone excretion in absence of glycosuria is always slight and the differences may fall within the limits of individual variation. This impression, nevertheless, is fairly positive and is based on many experiments.

Phlorizin glycosuria, as is well known, may be accompanied by heavy acidosis, but does not leave behind any tendency such as just mentioned in diabetic animals. In view of the sugar loss



TABLE 3  
Dog E5-37

Date 1917	Weight kg.	Urine					Blood Plasma			Remarks
		Vol. cc.	Sugar gm.	Total N gm.	Nitro- prusside Reaction	Total Acetone mg.	Sugar mg. per 100 cc.	CO <sub>2</sub> Cap. Vol. %	Total Acetone mg. per 100 cc.	
June 4	16.1	470	4.80	3.94	Heavy	405	.....	.....	....	Not fed.
5	15.6	650	Faint	3.50	Mod.	373	.....	.....	....	Not fed.
6	15.0	1000	Faint	2.52	Slight	290	.....	.....	....	Not fed.
7	14.5	No Urine	.....	.....	.....	.....	278	24.6	47	Autopsy blood. Muscle total acetone .031 %. Liver total acetone .061 %.

and the preponderance of fat in metabolism, attempts were made to start a heavy ketosis in this manner and then carry it further by high fat diets, but the acetonuria stops with the glycosuria, leaving the dog no more nor less disposed to ketosis than the normal. Epinephrin injections create so much gastrointestinal and general disturbance that fat feeding during this form of glycosuria is not feasible, but repeated epinephrin doses in fasting animals do not cause acetonuria. Mere sugar loss therefore does not produce ketosis when, as in epinephrin poisoning, the organism remains able to burn sugar freely.

Infections of various kinds create no tendency to acidosis in dogs.

Idiosyncrasy is an occasional highly important factor. The only two marked examples in the long series, embracing dogs of many kinds, but chiefly mongrels, were in Collies which seemed to be thoroughbred or nearly so. It is therefore a question whether the Collie race is specially susceptible to acidosis or whether these two examples were merely of accidental individual idiosyncrasy.

Dog E5-37 was an old, moderately obese female Collie weighing 20 kg. After being in stock for one week on bread and soup diet, she was partially depancreatized on May 14, 1917. The tissue removed weighed 36.1 gm., and the remnant about the main duct was estimated at 6 gm. (1/7). Early pregnancy was found in the operation, the fetuses being barely distinguishable by palpation.

The dog fasted following the operation, without glycosuria or ketonuria. May 29, when the weight was 17.15 kg., 4 gm. additional pancreatic tissue was removed, and fasting continued.

June 1, a trace of sugar appeared in the urine, and increased on June 2 to an excretion of 0.55 per cent. in 460 cc. of urine. A moderate nitroprusside reaction also appeared, and increased in parallel with the glycosuria. The dog also grew weaker and refused food.

The final record is shown in table 3. Death occurred with typical symptoms of fasting coma; namely, slight dyspnea and extreme prostration.

The gross autopsy was negative. No infection was discoverable. The muscles were in good condition, and large quantities of fat were still present under the skin and in the peritoneum. The microscopic examination showed merely the usual diabetic changes.

Dog D4-31 was a female Collie, aged 1 year and weighing 12 kg. in a medium or slightly thin condition. Fasting was begun October 7, 1916. The record is shown in table 4. For the sake of brevity, only sample days are shown.

Nitroprusside reactions remained negative during fasting, but quickly became heavy after a diet of 100 gm. beef-lung and 250 gm. suet was

TABLE 4

Dog D4-31

Date 1916	Weight kg.	Urine					Blood Plasma		Diet
		Vol. cc.	Sugar gm.	Total N. gm.	Nitro- prusside Reaction	Total Acetone mg.	Sugar mg. per 100 cc.	CO <sub>2</sub> Cap. Vol. %	
Oct. 4	10.7	48	Neg.	1.87	Neg.	---	---	---	Bread and soup.
5	---	120	Neg.	1.93	Neg.	---	---	---	Bread and soup.
6	---	No Urine	---	---	---	---	---	---	Bread and soup.
7	---	295	---	---	---	---	---	---	Fasting.
8	---	205	Neg.	8.82	Neg.	---	---	---	Fasting.
9	---	102	Neg.	4.67	Neg.	---	---	---	Fasting.
11	---	225	Neg.	3.09	Neg.	---	---	---	Fasting.
14	9.85	228	Neg.	9.51	Neg.	---	---	---	Fasting.
21	---	200	Neg.	9.07	Neg.	79	---	---	Fasting.
22	---	154	Neg.	3.36	Neg.	404	---	---	100 gm. lung and 250 gm. suet
23	---	216	Neg.	4.52	Heavy	308	---	---	100 gm. lung and 250 gm. suet.
24	---	395	Faint	4.28	Heavy	346	---	---	100 gm. lung and 250 gm. suet.
25	---	202	Faint	4.50	Heavy	162	119	53.8	100 gm. lung and 250 gm. suet.
26	---	144	Faint	1.51	Heavy	99	---	---	100 gm. lung and 250 gm. suet.
28	---	185	Faint	---	Mod.	15	---	---	100 gm. lung and 250 gm. suet.
30	---	162	Faint	2.15	Faint	48	---	---	100 gm. lung and 250 gm. suet.
Nov. 1	---	252	Faint	1.94	Faint	76	---	---	100 gm. lung and 250 gm. suet.
4	---	228	Faint	3.16	Slight	91	---	---	100 gm. lung and 250 gm. suet.
5	---	204	Faint	2.35	Slight	43	---	---	100 gm. lung and 250 gm. suet.
8	---	148	Faint	3.21	Slight	145	---	---	100 gm. lung and 250 gm. suet.
13	9.50	52	0.77	3.89	Mod.	185	100	38.5	100 gm. lung and 250 gm. suet.
21	9.80	58	0.28	1.15	Heavy	26	95	41.4	100 gm. lung and 250 gm. suet.
23	9.10	110	0.38	1.51	Slight	33	---	---	Fasting.
24	---	266	0.97	2.62	Slight	131	---	---	Fasting.
25	---	196	1.59	4.52	Heavy	138	98	46.6	1/2 pan bread and soup.
27	---	168	1.10	3.00	Heavy	130	---	---	1 pan bread and soup.
30	---	204	0.80	1.81	Slight	73	---	---	1 pan bread and soup.
Dec. 1	---	165	Faint	1.67	Heavy	102	---	---	150 gm. lung.
2	---	282	0.53	2.01	Heavy	114	---	---	150 gm. lung.
3	---	130	Faint	3.27	Neg.	0	---	---	150 gm. lung.
4	8.10	292	Faint	2.12	Slight	35	---	---	300 gm. lung at 4 p. m.
5	---	78	Neg.	4.00	Faint	87	---	---	
	---		Faint	---	Heavy	---	---	---	



begun on October 14. There was no vomiting or noticeable indigestion, though most of the suet had to be fed forcibly. October 23, a litter of premature pups were born, not viable. The same diet was continued.

The acetonuria reached rather high figures for a dog, and the plasma bicarbonate fell markedly. It was slightly higher on November 13 than on November 8, probably because of the loss of fat by vomiting and diarrhea on the intervening days. Fasting was begun November 21, chiefly because continuance of fat feeding was not feasible, but partly in order to learn whether fasting coma might develop. The nitroprusside reactions and quantitative acetonuria continued, but the plasma bicarbonate rose and there were no signs suggestive of coma, except weakness and lack of appetite.

On account of the threatening weakness, about half of a normal ration of bread and soup was fed forcibly on November 25, because the dog refused to eat anything. This program was repeated daily, without vomiting or diarrhea. Nevertheless, the acetonuria continued scarcely changed until December 2, when it finally became negative.

On December 1, diarrhea began, and the diet was therefore changed to 150 gm. of beef lung. Even this small meat ration had to be given forcibly. Acetonuria returned. By December 5, the weakness was profound, and the animal lay on her side unable to stand. An increased diet of 300 gm. beef-lung was forced. Death occurred in the early morning of December 6. Unfortunately, a blood sample taken in the final stage was lost, so there is no chemical evidence whether the death can be considered due to acidosis or not.

The autopsy showed nothing beyond emaciation. The liver, moderately fatty in appearance, weighed 368 gm.; both kidneys, 56 gm.; the pancreas only 12.1 gm. Microscopically, the liver was fatty, and there were vacuoles, probably representing fat, in numerous Henle tubule cells in the kidneys. The organs otherwise were negative.

It should be mentioned that the sugar reactions shown in the urine in table 4 were due largely or wholly to something else than glucose, because they persisted after fermentation. The reactions were also atypical, and the end-point in titration with Benedict's method was not clear. Similar conditions were encountered in a few other dogs on high fat diets. This dog was not diabetic, and the unique feature was the occurrence of serious or possibly fatal acidosis in a supposedly normal animal.

The possibility was mentioned that the Collie breed may be specially susceptible to acidosis. Some clinical writers state that pregnant women are abnormally liable to ketosis, and it will be noticed that the element of pregnancy was involved in both dogs, E5-37 and D4-31. The essential point is merely that these two dogs, for some reason, showed an extraordinary idiosyncrasy in regard to acidosis.

### *Summary and Conclusions*

1. Normal dogs ordinarily develop no more than trivial ketosis on fasting or high fat diets.

2. Preliminary carbohydrate feeding of any duration, obesity, preparatory deprivation of sugar by means of phlorizin, or epinephrin injections during fasting, create no increased susceptibility to ketosis.

3. The impression is stated that severely diabetic dogs, even when the sugar of blood and urine is kept thoroughly under control by diet, show a slightly increased liability to ketosis on high fat diets or fasting.

4. Two records of Collies are given, indicating that either this breed or these individual animals displayed a special idiosyncrasy with regard to acidosis.

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6. (4), table V.





## EXPERIMENTAL STUDIES IN DIABETES

### SERIES V.—ACIDOSIS

#### 4. *Acidosis in Puppies*

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Because of the prevalent belief that children are more subject to acidosis than adults, experiments with fasting and fat diets were performed on puppies. Their susceptibility to marked acidosis and early death was very striking, and search of the literature then revealed a statement of Blum, quoted by Neubauer,<sup>1</sup> that young dogs are more disposed to acetone excretion than older ones.

The experiments will be presented in two groups, namely: (1) normal puppies on fasting and fat feeding; and (2) pancreatectomy experiments.

##### 1. *Normal puppies on fasting and fat feeding*

Dog D4-38 was a mongrel male puppy, aged about 2 months, very strong and lively, weighing 1.9 kg. in medium nutrition.

Fasting was begun November 28. By December 2, only a trace of acetoneuria had appeared, and the pup was still obstreperously lively. On that day and the next, 75 gm. suet was fed and eaten ravenously. Ketonuria increased, as shown in Table 1. By December 4, the pup was weak and depressed out of proportion to the apparent nutrition. He ate 200 gm. beef lung voluntarily, but with poor appetite. December 5, the condition was worse, and bread and milk was fed, mostly by force, in an attempt to save the animal's life. The ketosis was appreciably diminished, though the plasma bicarbonate fell still lower, and death occurred in the early morning of December 6.

The gross and microscopic autopsy was negative. The emaciation was by no means extreme, and appreciable quantities of fat were still present.

Dog D4-40 was one of the same litter with Dog D4-38. Fasting was begun November 28, when the pup was in healthy condition at a weight of 1.4 kg. Ketonuria developed more rapidly than in dog D4-38, and the elimination of 92 mg. of total acetone on December 1 was large for such an animal.

December 2, the condition seemed to be safe, and 75 gm. suet was eaten voluntarily. Death occurred in the early morning of December 3,

TABLE 1  
Dog D4-38

Date 1916	Wgt. kg.	Urine					Blood Plasma			Diet
		Vol. cc.	Sugar gm.	Total N gm.	Nitro- prusside Reaction	Total Acetone mg.	Sugar mg. per 100 cc.	CO <sub>2</sub> Cap. Vol. %	Nitro- prusside Reaction	
Nov. 28	1.9	92	Neg.	1.01	Neg.	---	---	---	---	Not fed.
29	---	61	Neg.	1.02	Neg.	---	---	---	---	Not fed.
30	---	50	Neg.	0.96	Neg.	---	---	---	---	Not fed.
Dec. 1	1.9	24	Neg.	1.17	Faint	---	96	41.2	+	Not fed.
2	1.6	30	Neg.	0.62	Mod.	---	---	---	---	75 gm. suet.
3	---	24	Neg.	0.78	Mod.	25	---	---	---	75 gm. suet.
4	1.5	35	Neg.	0.82	Heavy	14	87	38.5	++	200 gm. lung.
5	---	40	Neg.	---	Heavy	12	---	32.1	+	Bread and milk.

TABLE 2  
Dog D4-40

Date 1916	Wgt. kg.	Urine					Blood Plasma			Diet
		Vol. cc.	Sugar gm.	Total N gm.	Nitro- prusside Reaction	Total Acetone mg.	Sugar mg. per 100 cc.	CO <sub>2</sub> Cap. Vol. %	Nitro- prusside Reaction	
Nov. 28	1.4	48	Neg.	1.01	Neg.	7	112	40.8	0	Not fed.
29	.....	41	Neg.	1.16	Slight	5	.....	.....	.....	Not fed.
30	.....	32	Neg.	1.05	Heavy	.....	.....	.....	.....	Not fed.
Dec. 1	1.3	32	Neg.	1.07	Heavy	92	.....	.....	.....	Not fed.
2	1.2	28	Neg.	0.89	Heavy	35	40*	21.7	++	75 gm. suet.

\* Autopsy.



preceded by the usual profound weakness and slight dyspnea. The blood analyses at the time of death are shown in table 2. Autopsy was negative as usual.

Dog F6-82 was a large Collie mongrel puppy, aged about one month. Fasting was begun May 21, 1918, when the pup was fat and strong at a weight of 1.3 kg. Acetonuria developed approximately as in dog D4-40. Also, the urine gave a distinct reaction and bright yellow precipitate with Benedict's copper solution, though the substance concerned was not glucose according to other tests.

May 28, the weight was still 1.05 kg., but the pup was weak and dyspneic. Besides the heavy ketonuria, a blood sample taken by needle puncture from the femoral vein showed plasma sugar 0.112 per cent.,  $\text{CO}_2$  capacity 19.5 volumes per cent.

The pup was thereupon returned to his mother in the hope that he might recover, but death occurred during the night. The gross autopsy was negative, and as the tissues were not fresh no microscopic examination was made.

Dog F6-97 was a female mongrel puppy, aged about 3 months. June 2, 1918, fasting was begun, when the nutrition and strength were good and the weight 2.3 kg. The record is shown in Table 3.

Ketosis was slower in onset and apparently milder than in the younger pups. By June 7, the nitroprusside reaction in the urine was heavy and the pup was becoming weak. The weakness increased until it reached a dangerous stage on June 10. On this day the pup was unable to stand and dimly conscious, and the respiration was gasping, deep and dyspneic. Rectal temperature  $36.7^\circ \text{C}$ .; pulse 200 per minute; respiration 16. The nitroprusside reaction in the plasma was negative, and the  $\text{CO}_2$  capacity was normal (53.8 vol. per cent.) in the morning.

At 11 A. M., 15 gm. glucose in 100 cc. water was given by stomach tube and retained. The morning hyperglycemia (0.161%) may possibly be attributed to the excitement of bleeding, but the subsequent marked glycosuria and hyperglycemia indicate some lowering of tolerance. The condition did not improve, and at 3 P. M. a subcutaneous injection of 4 gm. of glucose in 80 cc. 0.85 per cent. NaCl solution was given, in order to supply both food and fluid. As previously reported,<sup>2</sup> such measures have a powerful reviving effect in adult animals.

The acetonuria was cleared up but there was no return of strength. The plasma bicarbonate fell, as shown in the last blood sample, apparently not because of acidosis, but as either an agonal or a dilution phenomenon. Such dilution was scarcely proved by the percentage of corpuscles in centrifuged blood samples, but it is conceivable that bicarbonate might have passed into fluid retained in the tissues.

Death occurred from increasing weakness at 9 P. M. Autopsy was negative.



Dog F6-98 was a male puppy of the same litter with F6-97. June 2, he was in good nutrition at a weight of 2.1 kg., and fasting was begun. The record is given in Table 4.

Ketonuria developed as usual. By June 8, the pup was still fairly strong but without appetite. The low plasma bicarbonate of 27.1 volumes per cent. indicated danger more plainly than the clinical appearances. Suet 40 gm. was fed forcibly and retained, with talcum powder to prevent diarrhea.

June 9, the blood plasma was slightly turbid with fat, but the CO<sub>2</sub> capacity had risen to 30.0 volumes per cent. The pup was much weaker, evidently moribund. Glucose was given by stomach tube, but when left alone the pup vomited and, on account of weakness, aspirated the vomited fluid and died. Autopsy was negative as usual.

Dog G7-42 was a female mongrel puppy, aged about 2 months. June 26, 1918, she was fat and lively at a weight of 3.1 kg., and fasting was begun. Ketonuria developed as shown in Table 5.

July 1, there was a well marked nitroprusside reaction in the plasma as well as the urine, and the plasma bicarbonate was down to 36.6 volumes per cent. Nevertheless, the pup was strong and hungry, ate 75 gm. suet ravenously at 1 P. M., and continued lively and playful throughout the day. The slightly higher plasma bicarbonate at night, as compared with noon, may be due to the fact that there was some struggling with the taking of the noon blood, but none with the evening sample. There was slight visible lipemia in the 11 P. M. plasma, and a heavier nitroprusside reaction due to the digestion of fat. The most striking feature of the entire record is the hyperglycemia, which has been an occasional phenomenon accompanying fat-rich diets under various conditions.<sup>3</sup>

July 2, the pup was still strong and lively, and eagerly ate 75 gm. suet followed by 100 gm. beef-lung. The plasma bicarbonate that evening reached its lowest level of 32.8 volumes per cent., but the hyperglycemia was less pronounced.

July 3, the blood still showed visible lipemia in a sample taken 27 hours after the last feeding. The plasma bicarbonate had risen slightly to 38.5 volumes per cent., and the nitroprusside reactions in plasma and urine had become faint, evidently because of the protein fed along with the fat. Slight hyperglycemia persisted. Suet was refused, but 10 gm. lard, 100 cc. heavy cream and 50 gm. lung were taken fairly readily. Depression and weakness increased.

July 4, the pup was moribund, could not eat, and died when not under observation, so that a final blood sample was not obtained. Autopsy was negative.

Dog G7-43 was a male of the same litter as G7-42. He was in excellent condition, weighing 3.2 kg., when fasting was begun on July 26, 1918. The observations are shown in Table 6.



TABLE 5  
Dog G7-42

Date 1918	Wgt. kg.	Urine				Blood Plasma			Diet
		Vol. cc.	Sugar. gm.	Total N gm.	Nitro- prusside Reaction	Sugar mg. per 100 cc.	CO <sub>2</sub> Cap. Vol. %	Nitro- prusside Reaction	
June 26	3.1	28	Neg.	1.06	Neg.	.....	40.4	.....	Not fed.
27	.....	29	Neg.	1.82	Neg.	.....	.....	.....	Not fed.
28	.....	32	Neg.	.....	Slight	.....	.....	.....	Not fed.
29	.....	41	Neg.	1.23	Heavy	.....	.....	.....	Not fed.
30	.....	10	Neg.	.....	Slight	.....	.....	.....	Not fed.
July 1	.....	195	Neg.	1.28	Faint	145*	36.6	Mod.	75 gm. suet.
2	2.4	70	Neg.	0.48	Heavy	312†	38.2	Heavy	75 gm. suet and 100 gm. lung.
3	.....	95	Neg.	.....	Faint	175	32.8	Mod.	100 cc. heavy cream and 10 gm.
						149	38.5	Faint	lard and 50 gm. lung.

\* 12:30 P. M. † 11 P. M.

TABLE 6  
Dog G7-43

Date 1918	Wgt. kg.	Urine				Blood Plasma			Remarks
		Vol. cc.	Sugar gm.	Total N gm.	Nitro- prusside Reaction	Sugar mg. per 100 cc.	CO <sub>2</sub> Cap. Vol. %	Total Acetone mg. per 100 cc.	
June 26	3.2	22	Neg.	0.98	Neg.	.....	47.1	.....	Not fed.
27	.....	30	Neg.	1.27	Neg.	.....	.....	.....	Not fed.
28	.....	44	Neg.	2.14	Neg.	.....	.....	.....	Not fed.
29	.....	50	Neg.	1.34	Slight	.....	.....	.....	Not fed.
30	.....	94	Neg.	0.90	Heavy	.....	.....	.....	Not fed.
July 1	2.5	154	Mod.	.....	Slight	173	29.0	Mod.	11 a. m. 100 cc. 20% glucose by stomach.
2	.....	23	Faint	0.43	Faint	154	44.3	Faint	4 p. m. 100 cc. 20% glucose by stomach.

By the morning of July 1, the nitroprusside reaction had become heavy in the urine and moderate in the plasma, and the plasma bicarbonate was at the low level of 29.0 volumes per cent., though the pup still appeared very strong. Glucose feeding was tried, in order to learn whether the clearing of acetone in an animal still in apparently good clinical condition might save life in spite of the low plasma bicarbonate. Therefore 20 gm. glucose in 100 cc. water was given by stomach tube after taking blood at 11 A. M., and the same quantity at 4 P. M. The carbohydrate metabolism was disturbed, as shown by both hyperglycemia and glycosuria, but the glucose nevertheless resulted in a nearly complete disappearance of nitroprusside reactions from both plasma and urine.

July 2, the plasma bicarbonate had risen to 44.3 volumes per cent., but the pup was moribund. After the taking of the blood sample, an intravenous infusion of citrated blood from a normal dog was given, but neither the blood itself nor the alkali of the sodium citrate prevented death.

Dog G7-44 was a male of the same litter with the two preceding pups. He was in excellent condition at a weight of 3.0 kg. when fasting was begun on June 26, 1918.

By July 2, the nitroprusside reaction in urine and plasma was heavy, and the plasma bicarbonate was down to 31.9 volumes per cent. After the taking of the first blood sample at 1 P. M., 1 gm. sodium bicarbonate in 20 cc. water was given by stomach tube and retained, though some diarrhea soon resulted. At 3 P. M., the same dose of bicarbonate was given intravenously. Throughout the entire day the pup was lively and hungry, though weak. No clinical benefit was evident from the bicarbonate, though it raised the  $\text{CO}_2$  capacity of the plasma slightly above normal.

July 3, the pup was weaker. The nitroprusside reactions in plasma and urine seemed even heavier than before. The plasma bicarbonate was still normal at 50 volumes per cent. After the taking of blood, the pup was coaxed to drink 30 cc. milk and eat 50 gm. lung.

July 4, the urine was contaminated with diarrheal feces, but the acetone reaction had almost disappeared and the pup was stronger. With mixed diet, the improvement continued to complete recovery.

Some value of the bicarbonate dosage is suggested, but not fully proved, as it is not impossible that some pups at this stage may recover with food alone. The experiment at least shows that recovery is sometimes possible when the plasma bicarbonate has fallen as low as 31.9 volumes per cent.

Dog G7-45 was a female of the same litter as the preceding, but smaller and weaker than the others. The weight was 2.3 kg. in a state of good nutrition when fasting was begun on June 26, 1918.

Ketosis developed in approximately the same manner as in the stronger members of the litter, but the plasma bicarbonate reached the lowest level found in this group; namely, 25.2 volumes per cent. on July 1.

After the taking of this blood sample, the pup, though very weak, ate 100 gm. of beef lung. All other food was refused, and at 3 P. M., another 100 gm. of lung was fed forcibly and retained. The remarkable hyperglycemia of 0.270 per cent. at 10 P. M. seems to indicate that the impair-

ment of carbohydrate metabolism caused by fasting is demonstrable even with protein feeding. The nitroprusside reaction in plasma and urine was radically cleared up, and the  $\text{CO}_2$  capacity of the plasma rose considerably. The strength was not perceptibly changed.

The pup appeared safe to leave through the night, but was found dead the next morning. The gross autopsy was negative, and no microscopic examinations were made.

Dog F6-73 was a very strong male puppy, aged about 1 month when fasting was begun on May 13, 1918. The records of three successive fasts are given in Table 9.

In the first, May 13-19, the usual marked ketosis and lowering of the plasma bicarbonate occurred. The pup's strength sufficed to carry him through, and he recovered rapidly and completely on mixed diet beginning May 20.

The second fast, June 16-22, was interrupted by the feeding of 250 cc. milk on June 19. The resulting marked hyperglycemia is significant of the lowered carbohydrate tolerance under these circumstances. The most important fact is that this single inadequate feeding sufficed both to maintain strength and to prevent acidosis, and the animal was in good condition at the close of the fast.

The third fast, July 14-20, was imposed with a view to learning whether repeated fasting creates any "immunity" to acidosis and its accompanying symptoms. The results, as they stand, are affirmative, since ketosis was slight or absent and the fall of plasma bicarbonate was less than in the first fast. This result is inconclusive, however, in absence of the further experiments which were contemplated and which were impossible to carry out.

One factor to be considered, apart from any habituation, as an explanation of the smaller ketosis in the later fast, is merely the advancing age. A number of experiments, which will not be reported in detail, were performed to learn the age limits within which puppies are susceptible to fasting acidosis. These experiments were inconclusive, because of the different degrees of acidosis in different animals. It can only be said that the sensitiveness seems to be greater as the animals are younger, and with advance of age the susceptibility gradually diminishes. Puppies not above 4 months, or preferably not above 3 months, are best suited to show severe acidosis. Above the age of 9 months the behavior begins to resemble that of an adult dog, though the degree of acidosis developed still varies with individual idiosyncrasy or unknown factors. Diet is not the determining influence, for puppies fed on nothing but bread and milk from the time of weaning still gradually lose their tendency to fasting acidosis with advancing age.



TABLE 7  
Dog G7-44

Date 1918	Wgt. kg.	Urine				Blood Plasma			Remarks
		Vol. cc.	Sugar gm.	Total N gm.	Nitro- prusside Reaction	Sugar mg. per 100 cc.	CO <sub>2</sub> Cap. Vol. %	Total Acetone mg. per 100 cc.	
June 26 27 28 29 30	3.0	47	Neg.	1.76	Neg.	.....	47.1	Neg.	Not fed.
		28	Neg.	1.06	Neg.	.....	.....	.....	Not fed.
		56	Neg.	2.05	Neg.	.....	.....	.....	Not fed.
		39	Neg.	0.92	Neg.	.....	.....	.....	Not fed.
		38	Neg.	0.90	Faint	.....	.....	.....	Not fed.
July 1 2	2.5	42	Neg.	1.55	Slight	94	35.7	Heavy	Not fed.
		62	Neg.	0.83	Heavy	141	31.9	Heavy	Not fed. 20 cc. 5% sodium bicar- bonate by mouth.
						178*	67.2	Heavy	20 cc. 5% sodium bicarbonate intravenously.
3 4	2.2	115	Faint	2.18	Heavy	208	50.0	Heavy	Fed 30 cc. milk and 50 gm. lung.
		.....	Neg.	.....	Faint	.....	.....	.....	

\* Blood at 11 P. M.

TABLE 8  
Dog G7-45

Date 1918	Wgt. kg.	Urine				Blood Plasma			Remarks
		Vol. cc.	Sugar. gm.	Total N gm.	Nitro- prusside Reaction	Sugar mg. per 100 cc.	CO <sub>2</sub> Cap. Vol. %	Nitro- prusside Reaction	
June 26 27 28 29 30	2.3	44	Neg.	1.52	Neg.	.....	42.4	.....	Not fed.
		36	Neg.	1.33	Neg.	.....	.....	.....	Not fed.
		48	Neg.	.....	Faint	.....	.....	.....	Not fed.
		34	0.18	1.56	Heavy	.....	.....	.....	Not fed.
		52	Faint	.....	Heavy	.....	.....	.....	Not fed.
July 1	1.8	52	Faint	1.32	Heavy	139	25.2	Heavy	11 a. m. blood drawn. Fed 100 gm. lung.
		21	Slight	.....	Slight	270	37.6	Neg.	3 p. m. fed 100 gm. lung. 10 p. m. blood drawn.

TABLE 9  
Dog F6-73

Date 1918	Wgt. kg.	Urine				Blood Plasma			Remarks
		Vol. cc.	Sugar. gm.	Total N gm.	Nitro- prusside Reaction	Sugar mg. per 100 cc.	CO <sub>2</sub> Cap. Vol. %	Nitro- prusside Reaction	
May 13 14 17 18 19	1.5	---	---	---	Neg.	120	43.7	Neg.	Not fed.
	---	---	---	---	Neg.	---	---	---	Not fed.
	---	---	---	---	Neg.	---	---	---	Not fed.
	---	22	Neg.	---	Faint	---	---	---	Not fed.
	1.0	23	Faint	---	Heavy	115	32.8	Mod.	Not fed.
June 16 17 18 19	2.1	49	Neg.	1.95	Neg.	---	42.8	---	Not fed.
	---	32	Neg.	1.18	Neg.	---	---	---	Not fed.
	---	38	Neg.	1.20	Neg.	---	---	---	Not fed.
	---	43	Faint	---	Neg.	104	44.3	Neg.	3:30 p. m. blood drawn. 250 cc. milk.
	---	---	---	---	---	---	---	---	Fed
July 14 16 17 18 19 20	1.8	92	Neg.	3.22	Neg.	208 159	44.3 52.8	Neg. Neg.	8:30 p. m. blood drawn. Fed 150 gm. lung.
	2.6	---	---	---	---	156	45.3	Neg.	Not fed.
	---	67	Neg.	1.96	Neg.	---	---	---	Not fed.
	---	290	Neg.	---	Neg.	---	---	---	Not fed.
	---	175	Neg.	1.36	Slight	---	---	---	Not fed.
	2.0	90	Neg.	---	Neg.	---	---	---	Not fed.
	---	---	Neg.	---	Faint	116	38.4	Faint	Not fed.

The observation in the second fast of dog F6-73 is a sample of several which need not be detailed. Occasional inadequate feedings with carbohydrate or protein serve to prevent both ketosis and the early death associated with it. The animals merely waste away as in ordinary subnutrition. The acidosis condition is therefore clearly connected with fasting or fat diet.

Similar observations were made on several pups dying from incidental causes. A three months pup, which had taken or absorbed very little food for several days, died of a condition which was shown by the autopsy to be intussusception. A blood sample taken just before death showed the low plasma sugar of 0.079 per cent. and the high plasma bicarbonate of 59.5 volumes per cent., with no acetone in blood or urine. Puppies with fatal distemper are known to stop eating for some days before death, and have diarrhea and sometimes vomiting. Several of these, when examined near death, have been free from ketonuria and have had approximately normal plasma bicarbonate values. Puppies dying of cachexia from operative or other causes have shown no ketosis, and sometimes high and sometimes low plasma bicarbonate. For example, a ten-months pup, which developed cachexia instead of diabetes after partial pancreatectomy, emaciated to death in 16 days on account of poor appetite and digestion, and was free from ketosis throughout. The plasma at death contained 0.10 per cent. sugar, 35.3 volumes per cent. bicarbonate, and no acetone. The fact is now generally accepted that a reduction of plasma bicarbonate may be due to other causes than acidosis.

## 2. *Pancreatectomy Experiments*

As normal puppies are so highly subject to acidosis, it is natural to hope that diabetic puppies may offer an easy opportunity for the production of diabetic coma. The disappointment of this hope furnishes one of the paradoxes which trip up theorists in this subject.

Dog G7-32 was a female puppy of large mongrel type, aged about 2 months. The weight in good nutrition was 4.2 kg. June 20, 1918, the entire pancreas, weighing 14.3 gm., was removed. The subsequent observations are shown in Table 10.

On fasting, only trivial quantities of sugar were excreted. The nitrogen excretion was higher than in normal puppies. Marked ketonuria appeared within 24 hours but failed to increase. Very slight retention of acetone



TABLE 10  
Dog G7-32 (totally depancreatized)

Date 1918	Wgt. kg.	Urine				Blood Plasma			Diet
		Vol. cc.	Sugar. gm.	Total N gm.	Nitro- prusside Reaction	Sugar mg. per 100 cc.	CO <sub>2</sub> Cap. Vol. %	Nitro- prusside Reaction	
June 21	4.2	60	Faint	-----	Heavy	-----	-----	0	Not fed.
22	-----	63	Slight	3.36	Heavy	245	51.9	Slight	Not fed.
23	-----	72	Neg.	2.24	Slight	-----	-----	-----	Not fed.
24	3.3	45	Neg.	2.17	Heavy	85	50.0	Slight	Not fed.

TABLE 11  
Dog G7-48

Date 1918	Wgt. kg.	Urine				Blood Plasma			Diet
		Vol. cc.	Sugar. gm.	Total N gm.	Nitro- prusside Reaction	Sugar mg. per 100 cc.	CO <sub>2</sub> Cap. Vol. %	Nitro- prusside Reaction	
June 25	1.9	19	Faint	-----	Faint	-----	-----	-----	Not fed.
26	-----	130	1.44	1.40	Heavy	-----	-----	-----	Not fed.
27	-----	30	0.94	0.88	Heavy	-----	-----	-----	Not fed.
28	-----	40	1.33	1.27	Heavy	435	48.1	Slight	Not fed.
29	-----	37	0.25	1.02	Heavy	-----	-----	-----	Not fed.
30	-----	10	Neg.	0.52	Mod.	-----	-----	-----	Not fed.
July 1	1.4	52	Faint	1.47	Heavy	178	51.9	Heavy	50 cc. milk and 50 gm. lung.
2	-----	---	Neg.	-----	Slight	112	58.6	Slight	

was indicated by the plasma nitroprusside reactions, and the  $\text{CO}_2$  capacity scarcely changed. Cachexia dominated the picture, and death occurred in the early morning of June 25, with merely the same symptoms of weakness that are found in adult dogs.

Autopsy confirmed the completeness of the pancreatectomy and the absence of peritonitis.

Dog G7-48 was a female puppy, aged 6 weeks and weighing 1.9 kg. in very good nutrition. Total pancreatectomy was performed June 25, 1918. The subsequent record is shown in Table 11.

The animal bore the operation unusually well, and retained spirits as well as a normal fasting pup. Glycosuria diminished and disappeared, and the plasma sugar fell to normal at the end, as not infrequently happens in the final cachexia following total pancreatectomy. The ketonuria began earlier, but apparently was no heavier than in a normal fasting puppy. The plasma bicarbonate did not fall, and there were no symptoms of coma. The food given on July 1 was evidently not absorbed, as indicated by diarrhea and the absence of glycosuria or hyperglycemia. The terminal decline of ketosis was probably due to cachexia. The abdominal wound had appeared to be healing unusually well, but on July 2 one end of it was found separated, and death occurred from weakness and peritonitis at 10 P. M.

Autopsy confirmed the completeness of the pancreatectomy, and otherwise was negative except for emaciation and peritonitis.

If there is any difference between normal and depancreatized pups regarding acidosis, the latter are less susceptible. The reason—whether connected with increased protein catabolism, or cachexia—is unknown. In these weak little animals, glycosuria is soon suppressed by inanition. Fat feeding is not feasible because of the lack of digestive power. The survival after total pancreatectomy is shorter than in adult dogs, so that many such pups die before the number of days at which normal fasting pups develop their maximum acidosis. Older pups, with more strength to withstand the operation, are past the age for heavy fasting acidosis and react to pancreatectomy like adult dogs. Because of these circumstances, totally depancreatized puppies are a failure for purposes of acidosis.

Partial pancreatectomy is unsuccessful for similar reasons. Young puppies, at the best age for acidosis, quickly go into cachexia after the operation, especially with any attempts at fasting or fat feeding, and die with little or no ketosis. Older pups are no more susceptible to acidosis than adult dogs, and lack the endurance of the adults in withstanding either diabetes

or a program of heavy fat feeding. Coma has therefore never been produced in a diabetic pup.

An earlier publication<sup>4</sup> described the experience with diabetes in puppies, the production of which is more difficult and less satisfactory than in adult dogs. The records of the attempts at acidosis in the younger pups are not worth reproducing, as they show merely early cachexia and death. The protocols of only two of the older pups will be given as illustrations.

Dog D4-25 was born in the laboratory June 22, 1916. On September 15 he weighed 2.0 kg. in excellent nutrition, and was partially depancreatized. The tissue removed weighed 13.25 gm., and the remnant about the main duct was estimated at 2.7 gm. (1/6). Transitory glycosuria followed, but no lasting diabetes. The diet was bread, soup and milk, on which the pup grew and was fat.

November 28, fasting was begun as shown in Table 12. The nitroprusside tests of the urine remained negative for 4 days, then became heavy, and the quantitative acetone excretion was rather high for a dog of this size. By December 3, weakness was apparent but no signs of coma. Suet 100 gm. was eaten voluntarily, but though the acetonuria was higher on the following day, the nitroprusside test of the plasma was negative and the CO<sub>2</sub> capacity normal.

As the pup seemed too weak to withstand the program further and coma seemed unlikely, feeding was begun December 4, and he was later used for other purposes. A point of interest in the present connection is the slowness with which acetonuria cleared up on the carbohydrate diet.

The record of the 7-months pup D4-21 has been previously summarized.<sup>4</sup> September 15, 1916, permanent diabetes was produced by removal of eight-ninths of the pancreas. Subsequently, glycosuria was occasionally provoked by bread feeding, but was mostly kept under control by carbohydrate-free diet, often including much fat. In order that the animal should thrive, protein ordinarily predominated in such diets, but the record of an attempt to produce acidosis by a one-sided fat diet followed by fasting is given in Table 13. The pup was exceptionally resistant to ketosis, even more so than the average adult dog.

As mentioned in the previous description,<sup>4</sup> the animal with very slow downward progress developed heavy glycosuria and ketonuria at an adult age in 1918, and could doubtless easily have been sent into coma had circumstances permitted.

### *Discussion*

Acidosis is a biological phenomenon which must be studied as broadly as possible if it is to be adequately understood. Observations limited to a narrow group of conditions are apt to furnish insecure support for general conclusions. The ketosis of dogs and puppies is valuable because of the possibilities of compari-



TABLE 12  
Dog D4-25

Date 1916	Wgt. kg.	Urine				Blood Plasma			Diet
		Vol. cc.	Sugar gm.	Total N gm.	Total Acetone mg.	Sugar mg. per 100 cc.	CO <sub>2</sub> Cap. Vol. %	Nitro- prusside Reaction	
Nov. 28	5.5	242	Neg.	3.12	Neg.	---	---	---	Not fed.
29	---	161	Neg.	1.71	Neg.	---	---	---	Not fed.
30	---	120	Neg.	1.25	Neg.	---	---	---	Not fed.
Dec. 1	4.8	106	Neg.	0.90	Neg.	---	---	---	Not fed.
2	---	82	Neg.	2.12	17	---	---	---	Not fed.
3	---	75	Neg.	1.85	38	---	---	---	100 gm. suet.
4	4.5	108	Neg.	2.46	66	141	50.0	Neg.	200 gm. lung and a little milk.
5	---	212	Faint	3.48	42	---	---	---	200 gm. lung and bread and soup and milk.
6	---	108	Neg.	2.38	22	---	---	---	200 gm. lung and bread and soup.
7	---	135	Neg.	2.44	Neg.	---	---	---	200 gm. lung and bread and soup.

sons between this species and the human, and because of the greater freedom offered by animal as opposed to clinical experiments. Had an opportunity been obtained for carrying out these experiments with the requisite assistance and facilities, considerable light might have been thrown upon the following three questions, which are now stated only as open problems.

1. Relation of conditions in fasting puppies to diabetic coma. —The basic facts are that young normal puppies deprived of all food for a few days develop acetone in urine and blood and die at a time when the visible reserves of fat in the various depots and protein in the muscles should seem to preclude death from simple starvation. Fat feeding aggravates this condition and carbohydrate or protein, even in sub-maintenance quantities, prevent it and prolong life if the condition is not too far advanced. If this disturbance is essentially of the same nature as diabetic coma, such puppies can offer a valuable opportunity for studying the condition free from the complicating factors of diabetes or glycosuria. The differences from human diabetic coma are that consciousness is not lost much before death, dyspnea is not marked, and the quantities of acetone are comparatively small. All these clinical peculiarities, however, are practically duplicated in the fasting "coma" of human diabetics or diabetic dogs, which may differ greatly from the usual clinical picture, and in the similar condition which clinicians have described as the "heart failure" type of acidosis death, but there is no doubt that all these types are essentially the same. The profound weakness, out of proportion to the apparent nutritive state, is common to the acidosis of puppies and the other forms mentioned, and so also is the lack of appetite and vomiting of food. The marked fall of plasma bicarbonate is a strong point of chemical resemblance, but it should be remembered that this is by no means a true index of acidosis and the fall of bicarbonate may be merely a feature of the moribund state. It may be significant that puppies do not show the bloody admixture of the feces, which is practically a constant accompaniment of diabetic coma in dogs; but all such dogs, whether the coma comes on during feeding or during fasting, have been subjected to prolonged overfeeding with fat, and the attendant indigestion may possibly be one factor in causing the melena. Since the fundamental nature of diabetic coma is unknown, this question of identity cannot be answered

TABLE 13  
Dog D4-21

Date 1916	Wgt. kg.	Urine				Blood Plasma			Diet
		Vol. cc.	Sugar. gm.	Total N gm.	Nitro- prusside Reaction	Sugar mg. per 100 cc.	CO <sub>2</sub> Cap. Vol. %	Nitro- prusside Reaction	
Nov.	20	160	Faint	2.65	Neg.	193	51.8	Neg.	100 gm. lung and 200 gm. suet.
	21	268	Faint	2.79	Neg.	-----	-----	-----	100 gm. lung and 200 gm. suet.
	22	360	Faint	2.34	Neg.	-----	-----	-----	100 gm. lung and 200 gm. suet.
	23	292	0.63	2.18	Neg.	-----	-----	-----	100 gm. lung and 200 gm. suet.
	24	262	Faint	1.52	Neg.	-----	-----	-----	100 gm. lung and 200 gm. suet.
	25	240	Faint	2.45	Neg.	-----	-----	-----	100 gm. lung and 200 gm. suet.
	26	142	0.65	2.58	Neg.	-----	-----	-----	100 gm. lung and 200 gm. suet.
	27	272	Faint	2.86	Neg.	-----	-----	-----	100 gm. lung and 200 gm. suet.
	28	206	Slight	1.83	Neg.	-----	49.8	Neg.	100 gm. lung and 200 gm. suet.
	29	358	Faint	1.47	Neg.	-----	-----	-----	Not fed.
Dec.	30	218	Neg.	1.46	Neg.	-----	-----	-----	Not fed.
	1	224	Neg.	0.69	Neg.	-----	-----	-----	Not fed.
	2	155	Neg.	1.05	Neg.	-----	-----	-----	Not fed.
	3	104	Neg.	0.89	Neg.	-----	-----	-----	100 gm. suet.
	4	225	Neg.	4.03	Faint	200	50.0	Neg.	200 gm. lung.



at this time. All that can be said definitely is that fasting puppies are subject to a condition of ketosis and early death, evidently due to a disorder of fat metabolism, and this condition merits study in connection with the general problem of acidosis.

2. Relation of clinical conditions to chemical findings.—Three conditions are available for comparison, namely human diabetic coma, diabetic coma in dogs (which no one having experience with it can doubt as being essentially identical with the human condition), and the uncertain terminal condition of fasting normal puppies. Here the differences between species may serve to throw some radically new light upon old problems. The acetone bodies have caught the fancy of most observers, because they are the one chemically tangible expression of the metabolic disorder, and because they are quantitatively very prominent in the cases regarded as most typical. The variability of acetone figures in different cases, however, is one of the reasons why diabetic coma has never been satisfactorily explainable as a mere intoxication with acetone bodies. When we turn to dogs, both in diabetic coma and in the fasting ketosis of puppies, we find the acetone bodies much less prominent than in man. If human patients can survive the large quantities of ketones sometimes found in the urine and blood of cases that recover, it is scarcely conceivable that such a strong animal as the dog should be fatally poisoned by mere fractions of these amounts. Likewise, the lowering of the alveolar  $\text{CO}_2$  tension or of the plasma bicarbonate has been viewed entirely as the result of the overwhelming of the body's alkali reserves by the flood of keto acids. But it is hard to understand how the dog, with its high resistance to acid poisoning, can be thus overwhelmed by the comparatively trivial quantities of such acids which are revealed by analyses of urine, blood or tissues. Whether due to an accident of individual idiosyncrasy or to some general difference, the fact is that the diabetic puppies of this series failed to show the same fall of plasma bicarbonate as the normal ones, though their ketonuria began earlier and was seemingly not less intense. There is no proof that this form of death in either man or dog is due to pure acidosis, or to the consequences of a sub-minimal concentration of blood alkali. By suitable bicarbonate dosage the plasma bicarbonate can be held rigidly at a normal level without averting the typical coma death. An example was given (Dog G7-44, Table

7) in which alkali administration was probably of some benefit to a puppy, as it is also probably beneficial to some extent in human coma, but the failures have been more numerous in both patients and animals, and it is now generally recognized that alkali holds only a minor place in therapy. The hypothesis of a tissue acidosis, exceeding that represented in the blood, is unsupported by existing facts; and against it may be set an equally unproved possibility that the fall of blood alkali is due not to acidosis, but to some "toxic" alteration of capillary permeability or tissue affinity for alkali. More probably conditions are mixed, and both acidosis and biological alterations are involved in varying degrees. Attention has been given to the possible occurrence in dogs of other acids than those familiar in man, but titrations of the urinary acidity in dogs with diabetic coma and in fasting puppies have excluded any important quantities of such acids. The increased ammonia excretion with diabetic ketosis received a simple interpretation under the theories of the school of Naunyn and Magnus-Levy, but this view of ammonia formation is today seriously questioned. The ketosis of dogs often seems to be accompanied by superabundant ammonia formation for defense against the small quantities of acetone bodies, which would cast further doubt upon the old theories of acidosis. The liability of cachectic dogs to cystitis, and the strict precautions necessary for obtaining urine free from fermentation, involve difficulties which could not be overcome under the conditions surrounding this research. For this reason the figures obtained for both acidity and ammonia of the urine have been regarded as too uncertain to publish, and nothing can be stated positively concerning this important point. The long familiar reduction of carbohydrate tolerance with fasting, and the more recently discovered hyperglycemia sometimes resulting from excessive fat diets in these puppies and in adult dogs and in human subjects, come into interesting relations with the enormous doses of insulin required for the control of glycosuria and hyperglycemia in the presence of severe ketosis.<sup>5</sup> Another point of similarity is found in the fact that when diabetic coma is too far advanced, insulin ordinarily fails to save life in either man<sup>5</sup> or dog,<sup>6</sup> even though acetone may be cleared up completely and the blood alkali kept at sufficiently high levels by bicarbonate dosage. Likewise, fasting puppies in the last stage are not saved by glucose or other treat-



ment which clears up their chemical signs of acidosis. In general, the evidence seems to favor the view that the fatal element in ketosis is some metabolic disturbance deeper than the chemical signs or traditional chemical theories.

3. Ketogenic-antiketogenic balance.—Much interest in the metabolic laws underlying ketosis has been aroused by the admirable and painstaking work of Shaffer,<sup>7</sup> which, if supported and extended as it deserves, may yet furnish the key to the problem. Shaffer's own recognition<sup>8</sup> that the conditions of ketosis are complex and influenced by biological variables goes far to harmonize differences of opinion. His followers have been less commendable, for they have set up alleged general laws on the basis of observations on a few individuals under arbitrary uniform conditions, instead of investigating as wide a range of normal and pathological states as possible. In an informal discussion a few years ago the writer was compelled to assume an isolated position, in maintaining that ketosis is governed, according to the most probable interpretation of existing evidence, not merely by the two known chemical factors, namely glucose versus fatty acids, but also by a third variable, namely the living organism, which does not necessarily or invariably deal with the same food mixtures in the same way under all normal and pathological conditions. Without a detailed review of the literature, some facts may here be enumerated which either disprove the notion of inflexible chemical ratios or else require explanation before anybody is justified in setting up such an inflexible law.

(a) It should first be recognized that the question is rather vague and difficult of investigation. Whether to choose as a standard a threshold of ketosis, consisting in the appearance of barely a few milligrams of acetone in excess of the normal, or to permit a slight ketosis and base judgment on conditions which alter this to an appreciable degree, is an uncertain question and the results under different standards are different. The readiness with which upholders of the strict chemical laws can vary their opinions between ratios of 1 to 1, 1 to 2, etc., strengthens the doubts concerning the infallibility of the law.

(b) There is at least one marked exception to the supposed law in the work of Higgins, Peabody and Fitz.<sup>9</sup> This accurately established exception in a normal individual is important, in view of the fewness of the total observations on which the law



is set up. An apparent idiosyncrasy in certain normal dogs was described in the preceding paper.

(c) Pathological states are more apt to furnish exceptions. There is a widespread clinical belief in a variable tendency to acidosis among diabetics, and in an abnormal susceptibility to acidosis on the part of febrile patients, pregnant women, etc. The fact is positive that different diabetic patients and dogs vary widely in their liability to lipemia,<sup>10</sup> and similar differences as regards acidosis cannot be denied without investigation.

(d) An extraordinary and frequently fatal form of acidosis associated with vomiting and other symptoms in children is described in a large literature. It is improbable according to the descriptions that either the early onset or the severity of the ketosis can be explained without assuming some special factor in these cases beyond what exists in normal children.

(e) Those who accept no evidence except respiratory analyses showing the actual materials entering into combustion should notice that complete determinations of this sort failed to explain the exception encountered in the above mentioned work of Higgins, Peabody and Fitz. Also, they should make sure that combustion is the sole governing factor. The writer has made observations which agree with the findings of Benedict and Osterberg<sup>11</sup> that the feeding of sugar or protein to phlorizinized dogs greatly reduces the ketosis, even though the extra glucose is quantitatively eliminated. It may be possible that the metabolism is changed by such feeding, so that different ratios of fat and carbohydrate are burned in a manner demonstrable by respiratory experiments, but the phenomenon should be investigated rather than ignored.

The two strongest evidences of the role of the living organism remain.

(f) Species seems to create a sharp difference. The dog seems to produce acetone less freely than man, not because different proportions of fatty acids and glucose are burned, but because the canine organism seems to deal with these mixtures differently than the human organism. This difference, if admitted, proves positively the existence of a biological factor.

(g) Age seems also to be a positive influence. The susceptibility of puppies to fasting ketosis contrasts with the practical immunity of adult dogs. This difference is probably not limited

to one species. Children are supposed to be more readily subject to acidosis than grown persons, and it is conceivable that a normal infant suddenly exposed to complete starvation might develop the same sort of condition as a puppy. It may be urged that the youthful organism carries considerable fat, and with its higher metabolism may use up its glycogen stores more quickly than an adult. Respiration experiments comparing the proportions of glucose and fatty acids burned by the fasting puppy and the fasting normal dog will be instructive, but not decisive. The comparison must be extended also to the totally depancreatized dog. The most remarkable fact is that ketosis is so much more marked in the fasting puppy, which loses no sugar, than in the totally depancreatized adult animal, no matter how high the D:N ratios or the total metabolism may be in the latter. Apparently only two alternatives are open: either the respiratory quotient is lower and the ratio of fatty acid combustion to glucose combustion is higher in the normal fasting puppy than in the depancreatized dog, or else the specific biological susceptibility to ketosis is so much greater in the youthful organism that it surpasses even the difference created by heavy glycosuria. Either of these facts would be important to establish, but the latter must be assumed as more probable according to existing knowledge. If this assumption is correct, the importance of the biological factor in ketosis is demonstrated.

### *Conclusions*

1. Young puppies are subject to marked ketosis and early death with fasting. The condition is aggravated by fat feeding and prevented by small quantities of carbohydrate or protein. It offers some analogies with diabetic coma, and may perhaps offer a convenient experimental means for studying the same essential condition without diabetes.

2. Hyperglycemia was observed incidentally in some animals with this form of intoxication due supposedly to abnormal fat metabolism, and a lowering of carbohydrate tolerance was found uniformly in this condition. In one instance this lowered tolerance seemed to be manifested also by hyperglycemia following the feeding of protein.

3. Totally or partially depancreatized puppies did not prove to be subject to diabetic coma as anticipated, because cachexia quickly suppresses both glycosuria and ketosis.

4. The difference between puppies and either normal or depancreatized adult dogs strengthens the probability of a specific biological factor in ketosis, beyond the mere proportions of glucose and fatty acids metabolized.

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## EXPERIMENTAL STUDIES IN DIABETES

### SERIES V.—ACIDOSIS

#### 5. *Acidosis in Phlorizinized Dogs*

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Phlorizin poisoning offers a specially favorable opportunity for the study of acidosis and the factors controlling it, because this form of acidosis seems to result strictly from loss of carbohydrate under controllable conditions. Von Mering<sup>1</sup> and Lusk<sup>2</sup> have described the limp and semi-conscious state in which fasting phlorizinized dogs often die, and the ketosis of such animals has become familiar. Nevertheless, experienced investigators are aware that phlorizin ketosis is a variable and uncertain condition, and the laws of its appearance and disappearance have not been satisfactorily established.

Baer<sup>3</sup> has stood almost alone in the attempt to learn these laws. He concluded that acidosis accompanies nitrogen loss. A phlorizinized dog excretes no acetone as long as he is in nitrogen equilibrium, but develops ketonuria when the nitrogen balance becomes negative, even though the actual excretion of both nitrogen and sugar remains the same. Also sugar prevents acidosis even though it does not entirely prevent nitrogen loss. Baer therefore suggested that protein groups, which are easily lost from the body protein in sugar formation, but also easily restored, possess a specific significance for preventing acidosis, in the sense that they either prevent the formation or favor the combustion of acetone bodies.

This connection between ketosis and nitrogen loss, which had been a prevalent theory before the time of Baer's paper, has been almost universally discarded in favor of the simple doctrine that ketosis results from a lack of sugar in metabolism. Baer's observations, however, are closely related to the more recent finding of Benedict and Osterberg,<sup>4</sup> that protein feeding greatly reduces the ketosis of phlorizinized dogs even though the entire sugar

content of the protein is lost in the urine. Theories of ketosis are incomplete unless they take account of these facts.

This study was an attempt to learn something of the character and conditions of phlorizin ketosis. The urines were cage specimens collected with the usual precautions, without catheterization. Benedict's copper solutions were used for qualitative and quantitative urinary sugar tests, Benedict's picric acid method for plasma sugar, and Van Slyke's methods for plasma bicarbonate and for acetone bodies in urine and plasma. Both normal and diabetic (partially depancreatized) dogs were used, and were given subcutaneous injections of powdered phlorizin suspended in oil. The following are some sample records:

### 1. *Non-diabetic Dogs*

Dog C3-61, a male bull terrier, aged 5 or 6 years, and weighing 13.5 kg. in good nutritive condition, was partially depancreatized on February 23, 1916. The tissue removed weighed 23.6 gm. The remnant about the main duct was estimated at 11.4 gm. No diabetes resulted, but the dog became somewhat cachectic and by April 3 had emaciated to a weight of 11.9 kg. Strength and liveliness were still retained. The subsequent period of fasting and phlorizin is represented in Table 1.

Ketonuria was only moderate, and the plasma acetone was low and fell progressively to the end. By April 6, the animal seemed somewhat dazed, and leaned against the cage for support in standing. The condition progressed until on April 9 the dog lay on his side, dyspneic, semi-conscious, and unable to stand. The urine was spoiled by admixture with thin bloody feces. The rectal temperature was 100.5° F. Convulsions occurred occasionally. Glucose was administered as shown in the table. The ketosis was nearly cleared up, but the plasma bicarbonate was not raised nor the clinical condition improved. Death occurred at 2 P. M. The autopsy was negative.

Remarks. (a) This dog contrasts with C3-72. Perhaps because of the higher D:N ratios, the ketonuria was distinctly higher in this emaciated animal than in the enormously obese dog. (b) With the higher ketosis, the plasma bicarbonate did not fall as low as in C3-72. (c) As usual in the terminal stage, glucose failed to save life or raise the plasma bicarbonate, though the ketosis was largely cleared up.

Dog C3-72 was a spaniel, aged apparently 8 or 10 years, and enormously obese. The weight was 20.9 kg., though the normal weight could not have been above 10 kg. The urine constantly contained albumin and casts. Nevertheless, the animal was strong and lively. The experimental program is shown in Table 2.

TABLE 1  
Dog C3-61

Date 1916	Wgt. kg.	Urine						Blood						Phlo- rizin gmi. *	Remarks		
		Vol. cc.	Sugar gm.	Total N gm.	D:N Ratio	Nitro- prusside Reaction	Acetone and Diacetic Acid mg.	B-oxy. (as ac- etone) mg.	Total Acetone mg.	Sugar mg. per 100 cc.	CO <sub>2</sub> Cap. Vol. %	Nitro- prusside Reaction	Acetone and Diacetic Acid mg. per 100 cc.			B-oxy. (as acetone) mg. per 100 cc.	Total Acetone mg. per 100 cc.
Apr. 3	11.9	555	25.5	4.16	6.23	Neg.	44.4	17.4	61.8	154	46.2	Neg.	16.4	32.6	49.0	1	Not fed.
4	.....	610	26.5	6.52	4.07	Faint	21.4	28.6	49.6	93	50.0	"	13.0	lost	.....	1	"
5	11.0	1300	24.1	7.80	3.09	Heavy	60.0	35.8	95.8	86	46.2	Faint	.....	.....	32.8	1	"
6	.....	1585	26.4	8.97	2.88	"	49.6	127.2	176.8	80	44.7	"	15.0	13.3	28.3	1	"
7	.....	1531	27.6	8.77	3.09	"	.....	.....	.....	70	42.8	"	.....	.....	.....	1	"
8	.....	1899	13.2	4.99	2.65	Mod.	.....	.....	289.4	100	48.1	Slight	.....	.....	.....	1	"
9	.....	.....	.....	.....	.....	.....	.....	.....	.....	147	32.8	.....	.....	.....	21.0	.....	"
.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	250	30.9	.....	.....	.....	13.6	.....	"
.....	.....	.....	.....	.....	.....	Slight	.....	.....	.....	357	32.8	Neg.	.....	.....	6.7	.....	"

\* Subcutaneously.



The period of plain fasting, April 3 to 10, resulted in negative nitroprusside reactions and only trivial quantitative figures for acetone. The clinical condition remained excellent.

The subsequent phlorizin dosage, though not intended for "maximal" effects, probably gave rise to lower D:N ratios than would have been found in a normal animal. The acetone figures in blood and urine were likewise low. Nevertheless, the fall of plasma bicarbonate was approximately the same as in average animals which show much higher ketosis. Intoxication was somewhat slow in appearing, but began to be evident on April 20. On April 21, the dog was weak, scarcely able to stand, and so apathetic that when placed on his back with legs in air he would remain indefinitely without attempting to change position. On this day phlorizin was omitted, but 200 gm. suet fed forcibly. The ketonuria was increased on the two following days, but whether on account of the fat feeding or not is uncertain.

April 22 and 23, the condition remained practically the same except for the development of dyspnea and blood-tinged diarrhea. Urine specimens were lost or spoiled by contamination. April 24, the stertorous dyspnea and bloody diarrhea were increased, and the dog was slightly weaker. In a blood sample taken at 10 A. M., the plasma bicarbonate was 19.5 volumes per cent. The animal was then given 200 cc. of 10% glucose solution by stomach tube, repeated in 2 hours. Between these feedings, 300 cc. of 10% glucose solution was injected subcutaneously. The clinical condition was not altered. At 5 P. M., the plasma bicarbonate was down to 11.8 volumes per cent. Consciousness was not lost until the increasing weakness caused death at 11 P. M. The autopsy was negative except for slight interstitial nephritis.

Remarks. (a) The extreme obesity in this animal was attended not by high, but by unusually low ketosis. (b) The fall of plasma bicarbonate was fully as great as in any animals with much higher ketosis. (c) The intoxication was rather slow in onset, but proved fatal with the same symptoms as in animals with high ketosis. (d) Glucose administration in the terminal stage, though clearing up most of the ketosis, failed to check the fall of plasma bicarbonate or the progress to a fatal end.

Dog C3-54, a white bulldog, aged about 6 years and in excellent nutrition, weighing 20.2 kg., was subjected to fasting and phlorizin as shown in Table 3.

The acetonuria reached the unusual quantity of over 4 gm. daily. No great retention in the blood was indicated by the slight plasma nitroprusside reactions. The plasma bicarbonate fell progressively. Toxic symptoms were evident as early as March 17 and steadily increased. Vomiting began March 19 and continued to the end. Nevertheless, the animal showed both thirst and hunger up to March 22. Weakness was

TABLE 2  
Dog C3-72

Date 1916	Wgt. kg.	Urine						Blood						Phlo- rizin gm.	Diet			
		Vol. cc.	Sugar gm.	Total N gm.	D:N Ratio	Nitro- prusside Reaction	Acetone and Diacetic Acid mg.	B-oxy. (as ac- etone) mg.	Total Acetone mg.	Sugar mg. per 100 cc.	Hgb. %	CO <sub>2</sub> Cap. Vol. %	Nitro- prusside Reaction			Acetone and Diacetic Acid mg. per 100 cc.	B-oxy. (as acetone) mg. per 100 cc.	Total Acetone mg. per 100 cc.
Apr. 3	20.9	440	2.20	6.64	.....	Neg.	3.9	4.6	8.5	125	107	47.1	Neg.	.....	.....	.....	.....	Not fed.
4	250	Neg.	2.57	2.57	.....	"	.....	.....	.....	125	101	35.7	"	.....	.....	.....	.....	"
5	360	"	3.89	3.89	.....	"	.....	.....	.....	125	.....	48.5	Neg.	.....	6.3	.....	.....	"
6	19.6	570	"	1.84	.....	"	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	"
7	480	"	2.44	2.44	.....	"	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	"
8	651	"	2.49	2.49	.....	"	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	"
9	230	"	1.95	1.95	.....	"	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	"
10	18.5	150	3.27	1.28	2.55	"	8.5	10.7	19.2	94	.....	46.2	Neg.	9.0	16.0	25.0	.....	"
11	390	7.37	.....	.....	.....	"	.....	.....	.....	.....	.....	48.1	"	5.3	6.2	11.5	.....	"
12	418	6.68	4.10	4.10	1.60	"	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	"
13	234	4.05	1.89	1.89	2.66	"	.....	.....	.....	115	.....	48.1	Neg.	6.0	4.0	10.0	.....	"
14	483	8.21	3.82	3.82	2.20	"	.....	.....	.....	128	80	48.1	"	6.3	6.3	12.6	.....	"
15	759	3.79	4.89	4.89	.....	"	.....	.....	.....	125	.....	49.0	.....	9.1	Neg.	9.1	.....	"
16	397	5.85	3.66	3.66	1.62	"	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	"
17	333	4.66	2.46	2.46	1.87	"	10.7	13.3	24.0	85	74	47.7	Neg.	7.8	6.1	13.9	.....	"
18	17.1	742	6.83	5.42	1.25	"	23.7	38.6	62.3	139	88	41.4	"	2.7	4.4	7.1	.....	"
19	420	6.28	2.81	2.81	2.23	"	13.4	35.3	48.7	.....	.....	.....	.....	.....	.....	.....	.....	"
20	540	2.70	3.29	3.29	.....	.....	38.9	28.6	67.5	164	77	29.0	Neg.	3.1	2.8	4.9	.....	"
21	939	Neg.	2.44	2.44	.....	.....	.....	.....	.....	152	.....	29.0	"	7.7	11.8	19.5	.....	"
22	.....	.....	.....	.....	.....	.....	.....	.....	.....	218	.....	30.9	"	.....	.....	.....	.....	200 gm. suet.
23	.....	.....	.....	.....	.....	.....	.....	.....	.....	278	.....	19.5	.....	.....	.....	.....	.....	Not fed.
24	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	11.8	.....	.....	.....	.....	.....	10 a. m.
																		5 p. m.

progressive, but the dog became unable to stand only on March 23. During the last few days, feces slightly tinged with blood were passed, and some of the final urine samples were spoiled by contaminations.

On the final day of life, the dog remained conscious but absolutely limp and helpless. The rectal temperature was 102.6° F. The respiration was deep and unusually stertorous. The entire picture was typical of the canine form of coma. When the plasma bicarbonate had reached its minimum of 19.5 volumes per cent., successive injections of sodium bicarbonate were given intravenously, to a total of 300 cc. of 4% solution, raising the plasma bicarbonate to 60.5 volumes per cent. There was no apparent benefit. Between 10 and 11 P. M. convulsions occurred, probably as a result of the bicarbonate, and death occurred a few minutes after 11 P. M. The autopsy was negative.

Remarks. (a) The acetone bodies were specially prominent in this case, and the early death may be attributed either to them directly or to the deeper metabolic disorder of which they are an indication. (b) Alkali injections raising the plasma bicarbonate to normal failed as usual to save life.

Dog C3-69, a female mongrel, aged about 1 year, and in medium nutrition weighing 16 kg., was subjected to phlorizin and fasting as shown in Table 4.

The ketonuria was comparatively slight, approximately 0.5 gm. at its maximum. The plasma acetone concentration remained trivial. The plasma bicarbonate fell markedly. Depression and weakness were slightly evident as early as April 20. By April 8, the fully typical picture of canine coma was present. Dyspnea was only slight, but the dim consciousness was a more prominent feature than in most dogs. The dog was completely limp and helpless, the eye-balls soft, and the circulation so poor that the taking of blood samples was difficult. There was the usual blood-tinged diarrhea. The rectal temperature was 100.3° F., and rose to 101.8° with the glucose injections to be mentioned.

The attempt was made to learn whether any benefit might be obtained from abundant glucose to clear up acetone and abundant fluid to correct any dryness of the tissues and sweep out any possible poisons. Accordingly, beginning after the taking of the first blood sample at 10:30 P. M., 800 cc. of 10% glucose solution was injected intravenously in the course of an hour. Later, 200 cc. of the glucose solution and 800 cc. of 0.85% NaCl solution were injected subcutaneously. Ketonuria continued, but the slight ketonemia, as judged by the nitroprusside reaction of the plasma, was practically abolished. The plasma bicarbonate was not raised. After a brief strengthening effect, due to the increase of circulating fluid by the intravenous injection, the clinical condition showed merely the usual decline of strength to death at 7 P. M. There was the usual negative autopsy.

Remarks. (a) This record was chosen for contrast with dog C3-54. Here the acetone bodies were only a fraction of what they



TABLE 3  
Dog C3-54

Date 1916	Wgt. kg.	Urine						Blood Plasma				Phlo- rizin gm.	Remarks
		Vol. cc.	Sugar gm.	Total N gm.	D:N Ratio	Nitro- prusside Reaction	Acetone and Diabetic Acid mg.	B-oxy. (as ace- tone) mg.	Total Acetone mg.	Sugar mg. per 100 cc.	CO <sub>2</sub> Cap. Vol. %	Nitro- prusside Reaction	
Mar.	20.2	505	36.4	3.79	.....	Faint	25.3	146.5	171.8	.....	46.4	Neg.	Not fed.
13	.....	1240	36.0	8.53	4.27	Slight	148.8	384.4	533.2	108	.....	.....	"
14	.....	1000	50.0	10.44	4.78	Mod.	230.0	480.0	710.0	.....	.....	.....	"
15	.....	962	46.2	10.97	4.12	Heavy	298.2	442.5	740.7	.....	.....	.....	"
16	.....	1616	45.2	11.82	3.83	"	2713.0	1437.0	4150.0	137	42.4	Mod.	"
17	.....	1780	41.1	11.82	3.58	"	1726.6	2171.6	3898.2	.....	.....	.....	"
18	18.1	1395	44.6	9.25	4.68	"	2469.2	1646.1	4115.3	81	36.6	Slight	"
19	.....	110*	5.8	1.63	3.53	"	.....	.....	.....	.....	.....	.....	"
20	.....	1405	29.2	6.88	4.17	"	1357.1	2811.4	4168.5	91	30.0	Slight	"
21	16.0	338	21.2	6.08	3.48	Faint	.....	.....	.....	200	31.9	Neg.	"
22	15.3	52*	2.2	.....	.....	Faint	.....	.....	.....	100	30.0	Slight	"
23	14.7	67	1.8	1.51	1.83	Slight	.....	.....	.....	77	19.5	Faint	"
24	.....	170	5.4	.....	.....	.....	.....	.....	.....	.....	.....	.....	"
.....	.....	108	3.4	.....	.....	.....	.....	.....	.....	100	60.5	"	"
.....	.....	22	0.5	.....	.....	Faint	.....	.....	.....	.....	.....	.....	"

\* Partial specimen.

TABLE 4  
Dog C3-69

Date 1916	Wgt. kg.	Urine						Blood Plasma						Phlo- rizin gm.	Remarks
		Vol. cc.	Sugar gm.	Total N gm.	D:N Ratio	Nitro- prusside Reaction	Acetone and Diabetic Acid mg.	B-oxy. (as ace- tone) mg.	Total Acetone mg.	Sugar mg. per 100 cc.	CO <sub>2</sub> Cap. Vol. %	Nitro- prusside Reaction	Total Acetone mg. per 100 cc.		
Apr.	16.0	605	33.6	5.51	6.13	Slight	53.8	53.2	107.0	129	57.6	Neg.	.....	1	Not fed.
3	.....	620	35.8	10.60	3.59	Heavy	44.7	302.2	346.9	69	48.1	"	.....	1	"
4	14.9	900	37.5	13.86	2.71	"	64.8	215.0	279.8	95	42.8	Faint	.....	1	"
5	.....	1235	48.2	13.72	3.48	"	112.9	372.3	485.2	102	37.2	"	7.5	1	"
6	.....	920	43.7	13.68	3.19	"	98.4	458.5	556.9	112	39.5	Slight	10.7	1	"
7	.....	.....	.....	.....	.....	.....	.....	.....	.....	84	34.7	.....	.....	.....	"
8	13.2	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	"
.....	.....	115	6.1	0.68	.....	.....	4.6	3.9	8.5	800	29.0	Faint	.....	.....	"
.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	525	29.0	Neg.	4.8	.....	"
.....	.....	1253	71.5	1.54	.....	.....	11.3	135.1	146.4	.....	.....	.....	.....	.....	"

10:30 a. m. glucose injections began (see text).  
1 p. m. catheterized.  
2:30 p. m. blood drawn.  
5 p. m. blood drawn.  
7 p. m. urine to time of death.

were in that animal, yet the plasma bicarbonate fell in a somewhat similar manner, and intoxication and death occurred just as early and with practically identical symptoms. (b) Glucose and liberal quantities of fluid were ineffectual to save the animal in the terminal stage.

Dog F6-61, an old female bull terrier mongrel, slightly obese at a weight of 9 kg., was subjected to fasting and phlorizin as shown in Table 5. By April 26, slight weakness and depression were noticeable. These increased, and on April 29 vomiting began. The subcutaneous injection of 600 cc. physiological saline on April 19 gave no benefit. On May 1, the dog was very weak, still vomiting occasionally, and groaning at times as if with intestinal cramps. The rectal temperature was  $37.7^{\circ}$  C., the pulse 150, the respiration 14, very deep and labored. Consciousness was retained, but the entire picture strongly resembled the typical fatal acidosis of dogs. The animal was found dead on the morning of May 2.

Remarks. (a) Occasional fasting phlorizinized dogs die in this unexplained manner. The phlorizin dosage was not high enough to warrant a supposition of "phlorizin poisoning," which has sometimes been the tentative diagnosis in the early deaths of dogs receiving 1 gm. or more per day. The D:N ratios were comparable to those of a totally depancreatized dog, and this example may therefore be cited as an exception to the previously mentioned rule<sup>5</sup> that pancreatectomy is more quickly fatal than the loss of an equivalent amount of sugar from some other cause, such as phlorizin. But such deaths are not only unusual, but they are also of different type. The depancreatized dog dies in cachexia, while the death of the phlorizinized dog is somehow connected with the ketosis. (b) The proof of this last statement is found in the usual acidosis symptoms, and especially in the fact that such deaths are prevented by any conditions (diets, renal disorder), which prevent the ketosis. Though these deaths therefore seem to belong under the general head of ketosis, a chemical reason for them is hard to find. The acetone excretion diminished as death approached, as often happens in such dogs. Also, this was chosen as an instance in which the plasma bicarbonate was never below normal, so that a retention of much acid in the blood or tissues is hard to assume. The simple loss of sugar might be conceived as fatal, but the form of death seems to be closely similar to that in fasting puppies,<sup>6</sup> which have no glycosuria.

TABLE 5  
Dog F6-61

Date 1918	Wgt. kg.	Urine							Blood Plasma CO <sub>2</sub> Cap. Vol. %	Phlo- rizin gm.	Remarks
		Vol. cc.	Sugar gm.	Total N gm.	D:N Ratio	Nitro- prusside Reaction	Acetone and Diacetic Acid mg.	B-oxy. (as acetone) mg.	Total Acetone mg.		
April 19	9.0	48	0	1.23	-----	Neg.	0	12.0	12.0	-----	Not fed.
20	-----	156	5.3	3.82	1.39	"	0	42.0	42.0	0.5	"
21	-----	260	18.7	7.29	2.57	Slight	12.0	147.0	159.0	-----	"
22	-----	228	15.8	5.64	2.80	"	0	24.0	24.0	0.5	"
23	-----	370	19.1	-----	-----	Mod.	-----	-----	-----	1	"
24	-----	300	10.5	6.24	1.68	Heavy	70.0	160.0	230.0	1	"
25	-----	240	11.8	4.56	2.59	Mod.	70.0	170.0	240.0	-----	"
26	-----	400	14.5	5.75	2.52	"	70.0	160.0	230.0	-----	"
27	-----	370	11.4	4.64	2.46	Slight	80.0	110.0	190.0	-----	"
28	-----	600	8.4	4.51	1.86	Mod.	-----	-----	-----	1	"
29	6.8	280	9.1	4.44	2.05	"	-----	-----	-----	-----	"
30	-----	150	7.4	3.56	2.08	Slight	70.0	10.0	80.0	-----	" 600 cc. saline subcane- ously.
May 1	-----	100	3.9	2.31	1.69	Mod.	-----	-----	-----	-----	Not fed.



Dog F6-20, in good nutrition at a weight of 26 kg., was subjected to fasting and phlorizin as shown in Table 6.

By January 3, the dog was slightly weak, but without dyspnea or lowering of the plasma bicarbonate. The feeding of 500 gm. beef-lung on two successive days practically abolished the excretion of acetoacetic acid, but the B-oxybutyric fraction was merely diminished. This same result in more marked degree followed the feeding of 250 gm. of air-dry bread on January 5.

Remarks. (a) The clearing up of aceto-acetic before B-oxybutyric acid is a fact which may deserve consideration in connection with theories as to which of these acids is formed primarily and which secondarily in metabolism. (b) The other noteworthy points are the same as in dog F6-21 (Table 7).

Dog F6-21, in good nutrition at a weight of 24.5 kg., was subjected to fasting and phlorizin as shown in Table 7.

By January 3, the dog was slightly weak, but there was no dyspnea or reduction of plasma bicarbonate. The feeding of 500 gm. of beef-lung on this and the following day restored approximately normal strength, and reduced but did not abolish the ketonuria. The feeding of 400 gm. of bread on January 5 did not completely arrest the ketonuria. The experiment was stopped at this point, on the assumption that the negative nitroprusside reaction marked the end of ketonuria, but the quantitative analyses then showed a persistence of B-oxybutyric acid.

Remarks. Explanations of these phenomena can be only hypothetical. (a) The reduction of ketonuria with protein feeding, with continuance of maximal D:N ratios, seems to indicate that the absolute quantity of protein metabolized plays some part, or that the non-carbohydrate moiety of protein is not isoketogenic with the quantity of fat which it replaces in metabolism. (b) The temporary persistence of ketonuria with protein and carbohydrate feeding may perhaps be due to the use of these materials for filling the depleted glycogen reservoirs rather than for immediate combustion. At any rate the strengthening effect upon the animal was very noticeable.

Dog G7-91 started fasting and received 1 gm. phlorizin on July 30, 1918. Another injection of 1 gm. phlorizin was given on July 31, but none on August 1. The subsequent record is shown in Table 8.

The dog was senile, probably between 10 and 15 years of age, but strong and slightly obese. The D:N ratios were not maximal, and acidosis remained practically absent notwithstanding higher phlorizin dosage than given to many dogs in this series.

August 18, the dog was still in good spirits, without serious weakness and without important ketosis or lowering of the plasma bicarbonate. A

TABLE 6  
Dog F6-20

Date 1917	Wgt. kg.	Urine						Blood Plasma			Phlo- rizin gm.	Remarks	
		Vol. cc.	Sugar gm.	Total N gm.	D:N Ratio	Nitro- prusside Reaction	Acetone and Diacetic Acid mg.	B-oxy. (as ace- tone) mg.	Total Acetone mg.	Sugar mg. per 100 cc.			CO <sub>2</sub> Cap. Vol. %
Dec.	26.0	300	0	5.01	-----	Neg.	0	50.0	50.0	-----	-----	-----	Not fed.
26		370	0	4.42	-----	"	0	Trace	Trace	-----	-----	-----	"
27		1120	55.20	12.48	4.42	"	111.0	410.0	520.0	-----	-----	-----	"
28	25.0	1500	53.25	16.80	3.17	Mod.	350.0	900.0	1250.0	-----	-----	-----	"
29		1200	44.40	11.76	3.78	Heavy	500.0	1200.0	1700.0	-----	-----	-----	"
30		1130	50.40	10.68	4.72	"	540.0	2040.0	2580.0	-----	-----	-----	"
31													"
1918													"
Jan.													
1		1250	39.65	11.57	3.43	"	420.0	1690.0	2110.0	-----	-----	-----	"
2		1200	45.36	12.00	3.78	Mod.	360.0	1200.0	1560.0	-----	-----	-----	"
3	21.8	2050	71.40	19.74	3.62	Slight	170.0	530.0	700.0	137	62.4	Heavy	500 gm. lung.
4		2000	62.20	18.40	3.38	"	Trace	680.0	680.0	-----	-----	-----	"
5	21.0	2170	92.40	8.58	-----	Faint	0	220.0	220.0	70	59.5	Neg.	250 gm. dry bread.

TABLE 7  
Dog F6-21

Date 1917	Wgt. kg.	Urine						Blood Plasma			Phlo- rizin gm.	Diet
		Vol. cc.	Sugar gm.	Total N gm.	D:N Ratio	Nitro- prusside Reaction	Acetone and Diacetic Acid mg.	B-oxy. (as acetone) mg.	Total Acetone mg.	Sugar mg. per 100 cc.	CO <sub>2</sub> Cap. Vol. %	
Dec. 26	24.5	200	0	-----	-----	Neg.	0	0	0	-----	-----	Not fed.
27	-----	340	0	8.40	-----	"	0	100.0	100.0	-----	-----	"
28	23.5	440	30.8	8.20	3.75	"	Trace	70.0	70.0	-----	-----	"
29	-----	370	24.6	9.20	2.67	Slight	60.0	150.0	210.0	-----	-----	"
30	-----	800	32.0	12.16	2.03	Mod.	140.0	330.0	470.0	-----	-----	"
31	-----	840	41.9	12.78	3.28	"	190.0	420.0	610.0	-----	-----	"
1918 Jan. 1	-----	900	30.2	12.60	2.39	Heavy	180.0	630.0	810.0	-----	-----	"
2	21.3	1070	34.7	13.09	2.65	"	280.0	840.0	1130.0	-----	-----	"
3	-----	1720	78.1	20.26	4.05	Slight	130.0	340.0	470.0	92	59.5	500 gm. lung.
4	-----	1440	68.3	18.60	3.67	Faint	Trace	300.0	300.0	-----	-----	"
5	21.0	1550	59.8	9.28	-----	Neg.	0	160.0	160.0	72	53.8	400 gm. bread.

TABLE 8  
Dog G7-91

Date 1918	Weight kg.	Urine					Blood Plasma			Phlo- rizin gm.	Diet
		Vol. cc.	Sugar gm.	Total N gm.	D:N Ratio	Nitro- prusside Reaction	Sugar mg. per 100 cc.	CO <sub>2</sub> Cap. Vol. %	Nitro- prusside Reaction		
Aug. 2	17.4	840	27.09	10.62	2.55	Neg.	---	---	---	1	Not fed.
3	---	245	8.80	2.28	3.87	"	---	---	---	---	"
4	---	375	11.36	5.40	2.07	"	---	---	---	1	"
5	---	500	17.15	7.50	2.29	Faint	---	---	---	---	"
6	---	900	26.64	12.69	2.10	Neg.	---	---	---	1	"
7	16.1	No Urine	---	---	---	---	---	---	---	---	"
8	---	900	19.62	12.42	1.58	Faint	---	---	---	1	"
9	---	No Urine	---	---	---	---	---	---	---	---	"
10	---	414	10.40	5.45	1.91	Faint	---	---	---	---	"
11	---	860	25.65	11.88	2.15	"	64	52.6	Faint	---	"
12	---	Urine	Lost	---	---	---	---	---	---	1	"
13	---	"	---	---	---	---	51	---	---	---	"
14	---	440	12.70	5.70	2.23	Neg.	---	---	---	---	"
15	---	300	6.33	3.63	1.74	"	---	---	---	---	"
16	---	400	11.84	3.62	3.26	"	---	---	---	---	"
17	---	350	Faint	4.96	---	"	---	---	---	---	"
18	14.3	170	"	2.80	---	"	100	49.3	Faint	---	"



pan of cooked oatmeal fed the next day was eaten ravenously, and recovery was quick and complete on this diet.

After a week, when glycosuria had ceased, the animal was killed for autopsy. The pancreas was somewhat shrunken and hardened, and microscopically showed moderate interlobular fibrosis with no apparent involvement of islands. There was also marked interstitial nephritis. The disease in these two organs was evidently spontaneous and of long standing. The other viscera were negative grossly and microscopically.

Remarks. In this experiment, spontaneous chronic nephritis was the probable cause of the comparatively low glycosuria and the practical absence of acidosis with phlorizin. It is specially noteworthy that with this absence of acidosis or ketosis in a chemical sense there was also a complete absence of the usual clinical symptoms.

Dog F6-22, weighing 13.6 kg. in medium nutrition on December 26, 1917, was treated with fasting and phlorizin as shown in Table 9. The last injection of phlorizin was given on January 2.

January 3, the dog was dangerously weak, barely able to stand, unable to eat. There was no dyspnea, and the plasma bicarbonate percentage was above normal. After the taking of the blood sample, a subcutaneous injection of 50 gm. glucose in 500 cc. physiological saline solution was given. It cleared up both the ketonuria and the nitroprusside reaction of the plasma, and the plasma bicarbonate fell to a slightly subnormal level. There was no clinical benefit, and the dog died from weakness without dyspnea shortly after the taking of the final blood sample in the morning of January 4.

Remarks. (a) The abnormally high plasma bicarbonate in such experiments may possibly be due to concentration, and the fall after glucose injection may be due to dilution of the blood. (b) It is not certain whether the weakness is due to the ketosis or merely to the loss of sugar. (c) The failure to save life, even when acidosis is abolished, is a phenomenon which has since become familiar in the insulin treatment of terminal stages of diabetic coma.

Dog F6-23, in good nutrition at a weight of 25.5 kg., was subjected to fasting and phlorizin beginning December 26, 1917, as shown in Table 10.

January 3, the dog was weak but not dyspneic. The plasma bicarbonate was at the high level of 78.6 volumes per cent. The feeding of 50 gm. glucose on two days then cleared up the heavy ketonuria and restored nearly normal strength. The plasma bicarbonate fell to a slightly subnormal level. Clinical recovery was quick and complete on the subsequent bread diet.

TABLE 9  
Dog F6-22

Date 1917	Wgt. kg.	Urine							Blood Plasma			Phlo- rizin gm.	Remarks
		Vol. cc.	Sugar gm.	Total N gm.	D:N Ratio	Nitro- prusside Reaction	Acetone and Diacetic Acid mg.	B-oxv. (as ace- tone) mg.	Total Acetone mg.	Sugar mg. per 100 cc.	CO <sub>2</sub> Cap. Vol. %	Nitro- prusside Reaction	
Dec. 26	13.6	170	0	2.30	.....	Neg.	0	Trace	Trace	.....	.....	.....	Not fed.
27	.....	330	0	10.40	.....	"	52.0	64.0	116.0	.....	.....	.....	"
28	12.0	750	36.72	10.16	3.61	.....	0	140.0	140.0	.....	.....	.....	"
29	.....	1335	54.04	15.12	3.57	Faint	0	220.0	220.0	.....	.....	.....	"
30	.....	1250	48.10	14.56	3.31	Slight	180.0	490.0	670.0	.....	.....	.....	"
31	.....	1640	62.90	13.28	3.94	Mod.	310.0	530.0	840.0	.....	.....	.....	"
1918 Jan.	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
1	.....	1850	56.63	16.15	3.50	"	430.0	860.0	1290.0	.....	.....	.....	"
2	10.0	1800	64.00	15.12	3.57	Heavy	440.0	990.0	1430.0	.....	.....	.....	"
3	.....	730	24.00	3.12	.....	Faint	Trace	88.0	88.0	192	78.6	Mod.	50 gm. glucose subcutaneously.
4	9.8	.....	Heavy	.....	.....	Neg.	0	0	0	170	48.1	Neg.	Autopsy blood.

TABLE 10  
Dog F6-23

Date 1917	Wgt. kg.	Urine						Blood Plasma		Phlo- rizin gm.	Remarks	
		Vol. cc.	Sugar gm.	Total N gm.	D:N Ratio	Nitro- prusside Reaction	Acetone and Diacetic Acid mg.	B-oxy. (as acetone) mg.	Total Acetone mg.			Sugar mg. per 100 cc.
Dec. 26	25.5	1070	0	5.94	---	Neg.	---	---	---	---	---	Not fed.
27	---	470	0	4.80	---	"	---	---	0	---	---	"
28	23.6	910	29.60	8.48	3.50	"	Trace	300.0	300.0	---	---	"
29	---	760	26.40	9.76	2.70	Slight	88.0	250.0	370.0	---	---	"
30	---	1400	35.70	10.50	3.40	Mod.	420.0	1540.0	1980.0	---	---	"
31	---	1140	36.00	9.84	3.66	Heavy	440.0	1920.0	2360.0	---	---	"
1918	---	---	---	---	---	---	---	---	---	---	---	---
Jan. 1	---	1330	37.94	11.34	3.52	"	410.0	1400.0	1810.0	---	---	"
2	22.0	1550	36.00	9.60	3.75	"	420.0	1760.0	2180.0	---	---	"
3	---	1550	57.60	7.84	---	Faint	Trace	330.0	330.0	213	78.6	50 gm. glucose by mouth.
4	---	1720	54.00	5.04	---	"	"	220.0	220.0	---	---	"
5	23.2	1935	97.00	7.20	---	Neg.	0	0	0	108	49.0	800 gm. bread.
6	---	3000	60.90	12.30	---	"	---	---	---	---	---	"



Remarks. (a) Glucose abolished both ketosis and weakness in a phlorizinized normal dog. Therefore, either the weakness was due to the ketosis as such, without demonstrable loss of body alkali, or the ketosis and weakness were both independent results of the loss of sugar. (b) The hyperglycemia observed occasionally in this and other phlorizinized dogs in this series is not explained.

Dog F6-24, a very large strong male mongrel in good nutrition at a weight of 36.3 kg., was subjected to fasting and phlorizin as shown in Table 11. The acetone excretion was unusually high for a dog, but the fall of plasma bicarbonate was not in proportion.

By the evening of January 5, the powerfully built animal was too weak to stand. The rectal temperature was 38° C., the pulse 156, the respiration deep and sighing, the dyspnea being very well marked for canine acidosis. A blood sample at 6 P. M. showed only a slight reduction of plasma bicarbonate to 47.6 volumes per cent. The dog was then assisted to eat a pan of cooked oatmeal. An hour later, the condition seemed unchanged and the abdomen was ballooned out as if the digestive function were lacking. Therefore, 50 gm. glucose in 1 liter of physiological salt solution was injected subcutaneously. The succeeding urine samples showed almost immediate disappearance of acetone. By the next morning the dog was much stronger and acidosis entirely absent. Recovery was rapid and complete on carbohydrate diet.

Remarks. (a) In this experiment, the depletion of blood alkali was much less than in many dogs with far lower acetone excretion. (b) The dyspnea was out of proportion to the slight lowering of plasma bicarbonate. (c) In this normal dog, almost at the point of death from fasting and phlorizin, the acidosis was almost immediately cleared up and the symptoms relieved by carbohydrate.

Dog C3-49 was slightly obese at a weight of 18.75 kg. March 18, 1915, a diet of 300 gm. beef-lung and 500 gm. suet was begun, though it was seldom eaten completely. Subcutaneous injections of 1 gm. phlorizin in oil were given on March 18, 25, 29, and April 1, causing excretion of 25 to 35 gm. sugar per day. Nitroprusside reactions were negative in the blood plasma and only slight in the urine.

Complete fasting began April 1. The subsequent record, including the one additional phlorizin injection on April 3, is given in Table 12. The sugar excretion was practically unchanged. The D:N ratios were nearly "total," approximately the same as in the period of feeding. Nevertheless, the acetone rose immediately on fasting to considerable quantitative

TABLE 11  
Dog F6-24

Date 1917	Wgt. kg.	Urine							Blood Plasma		Phlo- rizin gm.	Remarks
		Vol. cc.	Sugar gm.	Total N gm.	D:N Ratio	Nitro- prusside Reaction	Acetone and Diacetic Acid mg.	B-oxy. (as acetone) mg.	Total Acetone mg.	Sugar mg. per 100 cc.		
Dec. 26	36.3	190	0	2.84	-----	Neg.	-----	-----	-----	-----	-----	Not fed.
27	No Urine											"
28	34.0	360	4.80	4.40	1.05	Neg.	Trace	76.0	76.0	-----	-----	"
29	-----	580	35.10	8.04	4.36	Faint	18.0	110.0	128.0	-----	-----	"
30	-----	1170	39.24	11.04	3.57	Slight	110.0	280.0	390.0	-----	-----	"
31	-----	800	29.60	6.88	4.30	Mod.	248.0	460.0	710.0	-----	-----	"
1918												
Jan. 1	-----	1150	35.28	8.88	3.97	Heavy	300.0	1560.0	1860.0	-----	-----	"
2	32.8	1680	41.14	11.39	3.61	Mod.	630.0	2210.0	2840.0	-----	-----	"
3	-----	1400	32.48	8.82	3.68	Heavy	670.0	2380.0	3050.0	213	57.6	"
4	-----	1840	31.16	8.55	3.64	"	703.0	2470.0	3173.0	-----	-----	"
5	31.3	2850	105.00	8.40	-----	Slight	450.0	1770.0	2220.0	94	47.6	"
												6 p. m. blood drawn; fed oatmeal and 200 gm. lung; 50 gm. glucose subcutaneously.
6	-----	2920	84.60	7.20	-----	Neg.	-----	-----	-----	98	51.7	"
												Ate oatmeal mixture and a little lung; drank a glucose mixture; 50 gm. glucose subcutaneously.

TABLE 12  
Dog C3-49

Date 1915	Wgt. kg.	Urine							Blood Plasma						Phlo- rizin gm.	Remarks	
		Vol. cc.	Sugar gm.	Total N gm.	D:N Ratio	Nitro- prusside Reaction	Acetone and Diacetic Acid mg.	B-oxy. (as ac- etone) mg.	Total Acetone mg.	Sugar mg. per 100 cc.	CO <sub>2</sub> Cap. Vol. %	Nitro- prusside Reaction	Acetone and Diacetic Acid mg. per 100 cc.	B-oxy. (as acetone) mg. per 100 cc.			Total Acetone mg. per 100 cc.
Apr. 3	15.1	1120	31.5	8.06	3.90	Heavy	276.8	276.0	552.8	105	32.8	Faint	32.4	74.0	106.4	1	Not fed.
4	-----	675	31.1	7.96	3.89	"	72.9	172.3	245.2	80	32.8	"	29.5	51.3	80.5	1	"
5	14.1	-----	27.6	-----	-----	Mod.	-----	-----	-----	193	30.0	Mod.	15.8	7.9	23.7	---	Urine spoiled by vomitus.
6	-----	Heavy	-----	-----	-----	Slight	-----	-----	-----	173	20.2	Heavy	-----	-----	2.25	---	Death.

values in urine and blood, and the plasma bicarbonate fell in 4 days from 32.4 to 20.2 volumes per cent.

The chief clinical symptom was weakness, which was noticeable especially after April 3. The dog refused food, but did not vomit until the last day. The prostration increased, so that walking or standing was difficult. A dazed mental condition and complete lack of interest in surroundings were present on the last day, but there was no unconsciousness. Dyspnea was slight on April 5, but marked on April 6, in the form of slow, deep, labored respirations. Death occurred apparently from profound weakness, as in shock.

Remarks. (a) The acidosis was more severe on fasting than on high fat diet. (b) The plasma acetone was not parallel to the plasma bicarbonate, but rather the reverse. (c) The nitroprusside reaction of the plasma was not an accurate index of the quantities of plasma acetone. (d) The total acetone content of the plasma, including both its acetoacetic and B-oxybutyric components, fell decidedly to the end instead of rising, and at death was about one-fifth what it had been on April 3. Though complete urinalyses were prevented by loss and contamination on the two closing days, a similar falling tendency is indicated in the urinary acetone.

Dog C3-97, a male mongrel, aged 2 or 3 years, with high strength and nutrition at a weight of 22.6 kg., was subjected to phlorizin injections and carbohydrate-free diets as shown in Table 13.

The animal was somewhat more resistant to ketosis than the average. The nitroprusside reactions in urine and blood plasma remained negative on the high fat diets up to June 20. Increasing quantities of acetone then developed in blood and urine with the fasting period up to July 4. (The low acetone output recorded for July 4 merely corresponds to the small volume of urine voided on that day, as the dog was not catheterized.) Slight symptoms of acidosis were evident at this time, and one or two days more of fasting would doubtless have brought a fatal result. Therefore, a diet of 600 gm. beef-lung was begun July 5. The symptoms were promptly relieved, and, though some ketosis persisted, there was a considerable fall in the acetone figures in blood and urine up to July 18. Beginning July 19, 100 gm. suet was added to this diet. The blood acetone did not increase, but the urinary acetone by July 26 had reached a higher figure than at any time during the fasting period.

At this time the dog began to appear unwell, in contrast to the former good condition. After July 28, food could not be taken and the urine samples were spoiled by contaminations. The partial specimens obtained indicated a fall rather than an increase of acidosis. Death occurred July 31, without acidosis symptoms. Autopsy showed purulent pneumonia of the entire right lung to be the cause of death. The other gross and



Dog C3-97

Date 1916	Wgt. kg.	Urine						Blood						Phlo- rizin gm.	Diet			
		Vol. cc.	Sugar gm.	Total N gm.	D:N Ratio	Nitro- prusside Reaction	Acetone and Diabetic Acid mg.	B-oxy. (as ac- etone) mg.	Total Acetone mg.	Sugar mg. per 100 cc.	CO <sub>2</sub> Cap. Vol. %	Hgb. %	Nitro- prusside Reaction			Acetone and Diabetic Acid mg. per 100 cc.	B-oxy. (as acetone) mg. per 100 cc.	Total Acetone mg. per 100 cc.
June	22.6	1230	10.09	7.38	1.37	Neg.	.....	.....	.....	81	52.1	112	Neg.	.....	.....	.....	.....	200 gm. lung and 300 gm. suet
12	.....	995	19.91	19.91	1.00	"	.....	.....	.....	122	.....	98	.....	.....	.....	.....	.....	"
13	.....	858	25.74	13.14	1.92	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	"
14	.....	525	19.43	5.87	3.31	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	"
15	21.8	605	21.78	9.38	2.30	.....	.....	.....	.....	122	.....	102	Neg.	.....	.....	.....	.....	"
16	.....	484	15.49	6.87	2.28	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	"
17	.....	564	6.77	7.58	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	"
18	.....	806	14.70	7.82	1.78	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	"
19	22.0	1112	16.68	7.83	2.20	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	"
20	.....	715	24.30	7.58	3.21	Neg.	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	"
21	.....	382	13.75	4.28	3.20	"	.....	.....	.....	93	.....	95	Neg.	.....	.....	.....	.....	"
22	.....	652	38.52	11.61	3.30	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	"
23	.....	No Urine	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	"
24	.....	425	20.40	9.40	2.13	.....	.....	.....	.....	74	46.2	104	Neg.	.....	.....	.....	.....	"
25	.....	690	32.71	12.56	2.62	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	"
26	.....	No Urine	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	"
27	.....	316	1.95	6.73	.....	.....	58.1	73.3	131.4	.....	.....	.....	.....	.....	.....	.....	.....	"
28	19.4	425	27.63	7.31	3.63	Mod.	91.8	119.0	210.8	.....	.....	.....	.....	.....	.....	.....	.....	"
29	.....	470	32.48	10.34	3.14	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	"
30	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	"
July	.....	No Urine	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	"
1	.....	354	22.30	9.17	2.41	.....	23.7	172.8	196.5	.....	.....	.....	.....	.....	.....	.....	.....	"
2	.....	968	43.56	14.65	3.15	.....	90.0	190.7	280.7	.....	.....	.....	.....	.....	.....	.....	.....	"
3	.....	196	9.99	2.43	3.22	Heavy	18.4	16.1	34.5	95	43.9	85	.....	21.5	75.9	97.4	.....	"
4	.....	796	44.58	12.42	3.56	"	98.7	61.7	160.4	87	38.1	109	.....	63.1	77.4	140.5	.....	"
5	18.2	740	46.25	16.13	3.15	.....	77.0	77.0	154.0	135	45.8	98	.....	52.0	45.8	.....	.....	600 gm lung.
6	.....	682	31.37	10.71	2.92	.....	70.9	57.3	128.2	109	42.9	99	.....	22.9	22.9	45.8	.....	"
7	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	"
8	18.8	1090	48.41	16.57	2.84	.....	82.8	91.6	174.4	127	39.1	78	.....	48.9	20.8	69.7	.....	"
9	.....	1040	44.30	16.22	2.74	.....	108.2	122.7	230.9	.....	.....	.....	.....	.....	.....	.....	.....	"
10	18.0	789	Heavy	.....	.....	.....	.....	.....	.....	97	.....	91	.....	47.5	16.3	63.8	.....	"
11	.....	1080	62.78	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	"
12	.....	620	35.46	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	"
13	.....	680	30.94	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	"
14	.....	800	41.04	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	"
15	.....	956	53.54	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	"
16	.....	582	37.83	12.46	3.02	.....	57.0	48.9	105.9	.....	.....	.....	.....	.....	.....	.....	.....	"
17	.....	1048	70.22	20.75	3.32	.....	95.4	102.7	198.1	.....	.....	.....	.....	.....	.....	.....	.....	"
18	.....	955	62.08	18.62	3.30	.....	57.3	45.8	103.1	131	38.5	84	.....	22.9	32.6	55.5	.....	"
19	.....	352	21.33	.....	.....	.....	21.5	35.2	56.7	139	32.8	74	.....	24.3	17.9	42.2	.....	"
20	17.6	860	53.75	17.20	3.12	.....	61.9	71.6	133.5	103	.....	92	.....	23.8	22.6	46.4	.....	"
21	.....	924	47.12	15.24	3.10	.....	51.7	71.1	122.8	102	.....	98	.....	32.4	28.1	70.5	.....	"
22	.....	1065	66.10	22.79	2.92	.....	74.6	110.8	185.4	.....	.....	.....	.....	.....	.....	.....	.....	"
23	.....	722	42.60	12.99	3.28	Heavy	43.3	109.7	153.0	.....	.....	.....	.....	.....	.....	.....	.....	"
24	.....	1384	73.35	21.77	3.34	"	116.3	202.8	319.1	.....	.....	.....	.....	.....	.....	.....	.....	"
25	.....	710	26.27	9.15	2.76	.....	107.9	193.1	301.0	118	.....	73	.....	17.3	21.7	39.0	.....	"
26	.....	1226	68.69	22.31	3.06	Heavy	53.9	333.5	387.4	.....	.....	.....	.....	.....	.....	.....	.....	"
27	.....	1290	54.18	18.82	2.83	"	35.0	165.1	200.0	.....	.....	.....	.....	.....	.....	.....	.....	"
28	17.0	968	32.23	.....	.....	"	.....	.....	142.3	128	30.0	.....	Slight	.....	.....	28.0	.....	"

microscopic findings in the organs were negative except for slight chronic interstitial nephritis.

Remarks. (a) The dog retained health, and practically held weight except for the fasting period and the undernutrition which was entailed by the diet of 600 gm. lung, together with phlorizin. This is another illustration of the different effects of phlorizin glycosuria and true diabetes.<sup>5</sup> (b) Fasting produces a more rapidly dangerous acidosis in phlorizinized animals than protein-fat diet. (c) Protein feeding reduces ketosis, but the addition of fat to the protein increases it. (d) Infection results in a decrease rather than an increase of ketosis in phlorizinized dogs, just as was formerly observed in diabetic dogs.<sup>7</sup> This is contrary to the well known effects of infection in human subjects. (e) The reduction of plasma bicarbonate here is hard to explain chemically, since the known power of acid excretion and ammonia formation in dogs should be expected easily to protect against the comparatively trivial quantities of acetone bodies.

Dog D4-00, male, normal, in medium nutrition, was subjected to phlorizin and protein-fat diet as shown in Table 14.

The diet was eaten well, except that the 150 gm. suet gradually proved to be too much and had to be reduced to 100 gm. on July 5. Ketonuria was moderate, similar to the figures in a diabetic dog, but not equalling the excretion of the ordinary fasting phlorizinized dog. The general condition remained good, until the diet was vomited on July 28, and weakness and depression were marked on July 29.

July 30, the weakness seemed dangerous, though the dog could still walk. The usual phlorizin dose was given in the morning, but toward noon it seemed advisable to take measures toward saving the animal's life. Therefore, 200 cc. of 30% glucose solution was injected subcutaneously, without apparent benefit. At 5 P. M., 100 gm. bread was fed forcibly. At 6 P. M., 400 cc. water containing 15 gm. glucose and 5 gm. sodium bicarbonate was given by stomach tube. Death occurred at 9 P. M. Dyspnea, unconsciousness, and the blood chemical changes of true coma were absent throughout.

Autopsy was immediate. The liver was very fatty and large, weighing 897 gm. The kidneys were small and dark red, weighing together 78 gm. Microscopically, the liver was extensively infiltrated with fat, but otherwise normal. The kidneys were normal except for marked edema of the epithelium of the glomerular capsules, and the usual vacuolation in Henle's loops. The pancreatic islands were fully normal in number, size and cytology. The acini varied in patches, being in some areas full of zymogen and in others empty and shrunken, as frequently found in cachexia. The other viscera were grossly and microscopically normal.



TABLE 14  
Dog D4-00

Date 1916	Urine										Blood					Phlo- rizin gm.	Diet
	Wgt. kg.	Vol. cc.	Sugar gm.	Total N gm.	D:N Ratio	Nitro- prusside Reaction	Acetone and Diabetic Acid mg.	B-oxy. (as ace- tone) mg.	Total Acetone mg.	Sugar mg. per 100 cc.	CO <sub>2</sub> Cap. Vol. %	Hgb. %	Nitro- prusside Reaction	Acetone and Diabetic Acid mg. per 100 cc.	B-oxy. (as acetone) mg. per 100 cc.	Total Acetone mg. per 100 cc.	
June 12	13.2	620	15.87	9.92	1.61	Neg.	.....	.....	.....	113	.....	100	Neg.	.....	.....	.....	200 gm. lung and 100 gm. suet
13	12	672	29.57	10.08	2.96	"	.....	.....	.....	87	.....	92	Neg.	.....	.....	.....	"
14	12.8	575	25.88	8.91	2.87	Faint	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	"
15	12.8	540	30.02	9.29	3.26	"	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	"
16	17	410	21.65	9.02	2.39	"	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	"
17	17	630	32.26	11.97	2.69	"	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	"
18	17	305	14.64	4.15	3.51	"	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	"
19	12.6	675	29.70	11.34	2.59	"	.....	.....	.....	89	.....	90	Neg.	.....	.....	.....	"
20	21	743	27.49	10.48	2.63	"	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	"
21	21	556	22.24	8.34	2.66	Neg.	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	300
22	22	640	26.69	10.69	2.49	Slight	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	"
23	22	640	19.90	6.44	3.08	"	.....	.....	.....	76	42.4	98	Faint	.....	.....	.....	"
24	24	812	30.04	11.69	2.57	"	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	"
25	25	308	14.17	6.34	2.20	"	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	"
26	26	370	19.50	9.32	2.09	"	.....	.....	.....	85	38.5	98	Neg.	.....	.....	.....	"
27	27	404	21.82	9.05	2.41	Mod.	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	"
28	12.6	405	26.33	9.39	2.79	"	32.4	108.1	140.5	.....	.....	.....	.....	.....	.....	.....	"
29	29	405	21.87	9.44	2.32	Heavy	42.1	34.4	76.5	.....	.....	.....	.....	.....	.....	.....	"
30	198	12.87	12.87	4.92	2.94	"	28.5	33.9	62.5	.....	.....	.....	.....	.....	.....	.....	"
July 1	645	22.58	7.61	2.92	2.92	"	86.4	73.5	159.9	.....	.....	.....	.....	.....	.....	.....	"
2	518	22.79	8.41	2.67	2.67	"	50.2	107.2	157.4	.....	.....	.....	.....	.....	.....	.....	"
3	870	21.23	9.40	2.26	2.26	"	108.1	116.6	224.7	.....	.....	.....	.....	.....	.....	.....	"
4	560	16.02	4.99	3.22	3.22	"	49.3	30.2	79.5	.....	.....	.....	.....	.....	.....	.....	"
5	13.3	865	25.09	8.49	2.92	"	70.9	76.1	147.0	93	.....	98	Mod.	57.5	60.0	117.5	"
6	336	34.98	7.39	2.48	2.48	"	36.3	29.5	65.8	.....	.....	.....	.....	.....	.....	.....	100
7	420	15.54	6.13	2.54	2.54	"	42.4	42.4	84.8	89	40.0	94	.....	.....	.....	.....	"
8	350	8.33	3.96	2.11	2.11	"	32.3	37.1	69.4	.....	.....	.....	.....	.....	.....	.....	"
9	758	26.59	12.13	2.23	2.23	"	97.0	106.6	203.6	.....	.....	.....	.....	.....	.....	.....	"
10	12.7	340	Heavy	7.44	2.47	"	41.2	41.2	82.4	112	40.0	97	Slight	16.7	11.9	28.6	"
20	12.8	496	18.35	7.44	2.82	"	50.1	61.2	111.3	99	.....	98	"	22.6	26.3	48.9	"
21	795	27.83	9.80	2.82	2.82	"	63.6	113.1	176.7	111	.....	92	.....	20.5	26.2	46.7	"
22	1010	26.77	8.56	3.12	3.12	"	4.3	8.1	12.4	.....	.....	.....	.....	.....	.....	.....	"
23	60	3.00	0.56	5.35	5.35	"	79.9	103.4	183.3	.....	.....	.....	.....	.....	.....	.....	"
24	1175	30.55	9.12	3.28	3.28	"	30.9	379.0	421.4	.....	.....	.....	.....	.....	.....	.....	"
25	758	19.71	6.19	3.24	3.24	"	42.4	42.4	84.8	112	.....	94	Mod.	25.7	29.5	55.2	"
26	515	14.42	4.43	3.24	3.24	"	30.9	148.3	179.2	.....	.....	.....	.....	.....	.....	.....	"
27	552	17.11	5.17	3.30	3.30	"	64.0	245.1	309.1	84	.....	78	Mod.	.....	.....	.....	"
28	11.5	550	12.76	.....	.....	"	.....	.....	.....	82	.....	115	Heavy	.....	.....	.....	"
29	.....	.....	.....	.....	.....	"	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	"
30	650	.....	15.54	.....	.....	"	28.6	82.7	111.3	66	39.8	102	Mod.	15.2	31.0	46.2	Glucose and bicarbonate (see text).



Remarks. Moderate phlorizin dosage, producing glycosuria equal to that of a severely diabetic dog, seems practically harmless on mixed diet, but cannot be endured indefinitely on a diet of limited protein and considerable fat. The cause of death is unknown. It is evidently not acidosis in the ordinary sense. The ketonuria of a phlorizinized animal is much less on protein-fat diet than on fasting. The symptoms and the blood picture of true coma, as found in diabetic dogs on fatty diets and in phlorizinized dogs on fasting, were absent in this and similar experiments. The plasma bicarbonate was not seriously reduced. There is no evidence that the acetone bodies were the cause of death, for they were at their highest in blood and urine in the early part of July, when the dog was in very good condition, and they fell markedly at the end. Simple hypoglycemia could scarcely be responsible for the death, for though the plasma sugar was 0.066% in the blood sample on July 30, the subsequent injection and feeding of carbohydrate gave no benefit. When given sufficiently early, carbohydrate has always saved phlorizinized dogs, but in this instance the unknown metabolic disturbance had evidently reached a fatal degree in which the injury was irreversible by treatment.

## 2. *Diabetic Dogs*

Dog D4-36, very well nourished at a weight of 16.5 kg., was partially depancreatized November 9, 1916. The tissue removed weighed 27.8 gm. The remnant about the main duct was estimated at 2.5 gm. (1/12). The resulting glycosuria was immediately checked by fasting and low protein-fat diet. With only 100 gm. beef-lung and 100 to 200 gm. suet, glycosuria remained absent, but it appeared when the lung was increased to 200 gm. on December 1, as shown in Table 15.

Slight ketonuria developed with the increasing glycosuria up to December 3, but was stopped by fasting. Beginning December 8, the diet was raised above the known tolerance, but phlorizin was begun December 10, in order to rob the body of more sugar than represented in the surplus diet.<sup>5</sup> It is thus probable that only phlorizin glycosuria was present and no active diabetes, but the death of the animal prevented a decision in this instance.

There was moderate acetonuria, but none of the clinical symptoms or blood chemical changes of coma. The dog seemed in fair condition, retained appetite, and had no dyspnea or striking weakness. The death on December 14 was therefore a surprise.

Remarks. (a) If this animal had survived a prolonged period of phlorization, and if on stopping phlorizin the tolerance had

TABLE 15  
Dog D4-36

Date 1916	Wgt. kg.	Urine					Blood Plasma			Phlo- rizin gm.	Diet
		Vol. cc.	Sugar gm.	Total N gm.	D:N Ratio	Nitro- prusside Reaction	Total Acetone mg.	Sugar mg. per 100 cc.	CO <sub>2</sub> Cap. Vol. %	Nitro- prusside Reaction	
Dec. 1	.....	316	Faint	6.23	.....	Faint	63.2	.....	.....	.....	200 gm lung and 100 gm. suet.
2	.....	356	1.78	8.97	.....	"	.....	.....	.....	.....	" " " " " "
3	.....	280	9.66	.....	.....	Slight	.....	.....	.....	.....	" " " " " "
4	14.6	280	1.68	5.01	.....	Neg.	.....	.....	.....	.....	Not fed.
5	.....	195	Faint	2.94	.....	"	.....	.....	.....	.....	"
6	.....	202	Neg.	3.49	.....	"	50.5	.....	.....	.....	100 gm suet.
7	.....	290	Faint	2.55	.....	"	.....	.....	.....	.....	"
8	.....	214	Neg.	2.63	.....	"	65.8	.....	.....	.....	200 gm. lung and 100 gm. suet.
9	.....	190	"	5.17	.....	Slight	47.5	.....	.....	.....	300 " " " " " "
10	.....	522	14.62	14.20	1.03	Faint	240.0	.....	.....	.....	" " " " " "
11	.....	872	46.22	10.46	4.42	Slight	566.8	111	48.6	Faint	" " " " " "
12	14.4	554	37.12	9.18	4.05	Heavy	98.0	.....	.....	.....	" " " " " "
13	.....	1035	54.86	.....	.....	Mod.	52.0	.....	.....	.....	" " " " " "
14	.....	398	17.12	5.04	3.40	Heavy	40.0	50	52.8	Slight	Died early in afternoon.

been found unimpaired, it would have been concluded that phlorizin glycosuria had given protection against true diabetes, according to the plan of experiment which was proved to be feasible in a former paper.<sup>5</sup> (b) It was known from other experiences that maximal phlorizin doses are inadvisable in the attempt to produce coma, because some dogs, particularly those with active or latent diabetes, die with what in our laboratory was vaguely called "phlorizin poisoning." As the plasma sugar of this dog on the morning of death was only 0.050%, it appears plausible from the recent Toronto work with insulin that some of these previously unexplained phlorizin deaths were due to hypoglycemia and had no connection with acidosis.

Dog D4-74, a black and white female mongrel weighing 15 kg. in a very good nutritive state, was partially depancreatized January 17, 1917. The tissue removed weighed 35.8 gm. The remnant about the main duct was estimated at 3.5 gm. (1/11). Heavy glycosuria ensued on bread diet and also on unlimited meat diet, but then was halted by two fast-days (February 2 and 3), and remained absent thereafter on a diet of 300 gm. beef-lung and 250 gm. suet. The weight, which had fallen as low as 12 kg., gradually rose on this regime to 13.1 kg., at the beginning of the period shown in Table 16.

In this period, the attempt was made to imitate the conditions of diabetes in a partially depancreatized dog, by giving small doses of phlorizin at such intervals that glycosuria was generally heavy and was never entirely absent. This program was continued through the interval, March 20 to April 1, for which the analyses are too incomplete to be included in the table. There was slight ketonuria, not unlike that of a dog with active diabetes on a similar diet, but the general picture was entirely different. The nitroprusside reaction in the blood plasma was negative; acidosis symptoms and tendency to coma were absent, and the dog gained weight and was in good condition at the termination of the experiment.

Remarks. This experiment was planned partly for another purpose, namely, to illustrate the difference between phlorizin glycosuria and diabetic glycosuria, even when equal quantities of sugar are lost.<sup>5</sup> To make the paradox stronger, a dog with potentially severe diabetes under control by diet was chosen for the phlorization. Unfortunately, no single dog with active diabetes is available for accurate comparison, and it can only be said from general experience that the strength and spirits are much better retained with phlorizin than with true diabetes. Likewise, a diabetic dog which has been made to gain weight by a month of high fat diet, and then allowed to develop this degree of



TABLE 16  
Dog D4-74

Date 1917	Wgt. kg.	Urine						Blood Plasma		Phlo- rizin gm.	Diet
		Vol. cc.	Sugar gm.	Total N gm.	D:N Ratio	Nitro- prusside Reaction	Total Acetone mg.	Sugar mg. per 100 cc.	Nitro- prusside Reaction		
Mar. 10	13.1	272	Neg.	3.89	.....	Neg.	62.6	84	Neg.	.....	200 gm lung and 400 gm. suet.
11	.....	1057	30.65	6.87	4.55	Faint	200.8	110	"	0.5	"
12	12.6	749	23.97	9.81	2.44	Mod.	365.0	57	Faint	.....	"
13	12.4	533	24.52	7.62	3.18	Heavy	106.6	.....	.....	.....	"
14	.....	472	7.08	7.50	0.95	"	108.6	.....	.....	.....	"
15	12.3	573	Trace	9.86	.....	"	194.8	.....	.....	.....	"
16	12.1	733	10.99	7.70	1.42	Doubtful	66.0	.....	.....	0.5	200
17	12.4	1182	22.46	13.83	1.65	Faint	94.6	.....	.....	.....	"
18	.....	470	13.91	7.19	1.94	Slight	75.2	.....	.....	.....	"
19	12.6	401	1.22	4.45	.....	Neg.	24.1	105	Neg.	.....	"
April 2	12.9	270	Trace	6.70	.....	"	45.0	133	"	.....	"
3	13.0	578	38.70	7.70	5.02	Faint	30.0	128	"	0.5	100
4	13.3	700	38.36	12.74	3.02	Mod.	336.0	.....	.....	.....	"
5	12.8	554	25.98	10.56	2.46	"	744.0	.....	.....	.....	"
6	12.9	385	1.85	8.90	.....	Faint	120.0	128	Neg.	.....	"
7	13.0	215	Trace	5.85	.....	Neg.	60.0	145*	"	.....	"
8	.....	382	4.45	8.30	.....	Slight	120.0	.....	.....	0.5	"
9	13.2	435	14.50	7.25	2.00	Neg.	.....	130*	Neg.	.....	"
10	13.3	370	10.88	8.48	1.28	"	.....	147	"	.....	"
11	13.2	352	4.64	7.80	.....	"	.....	.....	"	.....	"
12	13.5	352	18.12	.....	.....	"	.....	.....	"	.....	"
13	13.6	363	16.12	8.44	1.91	"	.....	79	"	.....	"

\* 6:00 P. M.

glycosuria for a month on the diet shown, will exhibit greater ketonemia and symptoms often terminating in coma. One positive statement seems warranted by the general experience: namely, that carbohydrate or protein added to the fat in the diet of a diabetic dog does not interfere in the slightest with the development of acidosis and coma<sup>8</sup>; but with phlorizin glycosuria no dangerous acidosis ever occurs when such quantities of carbohydrate and protein are given, even when the amount of sugar excreted is equal to that in diabetes.

Dog C3-98, a brindle male mongrel, aged 2 years, weighing 13.5 kg. in medium nutrition, was partially depancreatized June 8, 1916. The tissue removed weighed 25.8 gm. The remnant about the main duct was estimated at 2.1 gm. (1/13). The resulting diabetes was comparatively mild, for unlimited protein diet was tolerated and the addition of 100 gm. bread was necessary for slight glycosuria.

August 15, the former protein-carbohydrate diet was changed to 400 gm. beef-lung, 75 gm. bread and 100 gm. suet. September 7, phlorizin injections were begun as shown in Table 17. The loss of sugar, exceeding the carbohydrate of the diet, gave rise to slight ketosis. The dog remained well, ate the diet well, and had satisfactory digestion as attested by the nearly stationary weight. No tendency to persistence of ketosis could be revealed by the diet of 200 gm. lung and 200 gm. suet when phlorizin was discontinued after October 24.

From November 8 to December 20, the utmost effort was made to break down the tolerance by a diet of bread and soup with addition of first 200 and then 300 gm. of glucose daily. The animal was no longer diabetic and no glycosuria could be thus produced.

December 21, laparotomy showed the pancreas remnant to be two or three times as large as the mass left at the original operation. Tissue weighing 0.85 gm. was removed and was microscopically normal. The attempt to break down the tolerance was then repeated, with the result of slight glycosuria for a few days, but no lasting diabetes. February 8, 1917, additional pancreatic tissue weighing 0.5 gm. was removed. Carbohydrate overfeeding then brought on diabetes. The animal was used for other purposes, and died June 4 at a weight of 9.25 kg. The pancreas remnant, though reduced both by slight sclerosis and by the general emaciation, still weighed 2.95 gm.

Remarks. (a) No diabetic dog ever survives glycosuria of this degree on such a diet for such a length of time with practically unimpaired weight and strength. The great majority die of cachexia within a shorter period. This record is therefore a further illustration of the fact pointed out in a former paper,<sup>5</sup> that lack of insulin is far more serious than lack of carbohydrate.

(b) When diabetic dogs digest a diet of this kind sufficiently well to maintain their weight with glycosuria of this intensity, they invariably develop severe acidosis and die in coma (so far as the writer's experience extends) within a shorter time than shown in this table. This is one illustration of a general rule: namely, that phlorizinized dogs develop little or no acidosis on mixed diet, while mixed diet (so long as it includes sufficient fat) offers not the slightest hindrance to the development of fatal acidosis in a diabetic dog. (c) The complete independence of phlorizin glycosuria and true diabetes is further demonstrated by the fact, also described previously,<sup>5</sup> that there is no impairment of the diabetic tolerance with phlorizin, but rather the reverse. This dog was diabetic at the beginning of the experiment, and the tolerance could then have been broken down by overfeeding, notwithstanding hypertrophy of the pancreas remnant. After the period of phlorizin glycosuria, the dog was no longer diabetic. The pancreatic island function had not been injured by phlorizin, but on the contrary the loss of more sugar in the urine than was contained in the carbohydrate of the diet probably spared the island function so as to facilitate the recovery from the diabetes.

### *Summary and Conclusions*

It is evident that these observations touch only the surface of the problem, and a much deeper insight could have been obtained had there been adequate chemical assistance to permit taking advantage of the experimental conditions produced. The following features, however, may prove helpful to those who are able to pursue the subject farther.

1. Phlorization of fasting normal dogs under suitable conditions always gives rise to ketosis. With the high dosage (1 gm. per day or over) generally used for obtaining "total" D:N ratios there may be atypical deaths from unknown causes, possibly from direct poisoning, or from hypoglycemia or other conditions than acidosis. When smaller or less frequent doses are used, the renal injuries or other factors disturbing acidosis seem to be less prominent, and a condition which may be regarded as "coma" is obtained with fair regularity.

2. Different dogs differ considerably in the quantities of acetone bodies which they produce. These differences are not



TABLE 17  
Dog C3-98

Date 1916	Wgt. kg.	Urine					Blood						Phlo- rizin gm.	Diet			
		Vol. cc.	Sugar gm.	Total N gm.	Nitro- prusside Reaction	Total Acetone gm.	Sugar mg. per 100 cc.	CO <sub>2</sub> Cap. Vol. %	Hgb. %	Nitro- prusside Reaction	Acetone and Diacetic Acid mg. per 100 cc.	B-oxy. (as acetone) mg. per 100 cc.	Total Acetone mg. per 100 cc.				
Sept. 5	13.0	466	Faint	8.90	Neg.	.....	.....	.....	.....	.....	.....	.....	.....	400 gm. lung,	100 gm. suet and	75 gm. bread.	.....
6	.....	252	Neg.	4.09	"	.....	57	51.9	75	Neg.	.....	.....	.....	"	"	"	0.5
7	.....	525	27.00	.....	"	.....	.....	.....	.....	.....	.....	.....	.....	"	"	"	.....
8	.....	732	46.00	8.42	"	.....	.....	.....	.....	.....	.....	.....	.....	"	"	"	0.5
9	.....	700	58.00	18.94	"	.....	58	53.8	.....	Neg.	.....	.....	.....	"	"	"	.....
10	.....	670	51.90	20.51	Doubtful	.....	.....	.....	.....	.....	.....	.....	.....	"	"	"	0.5
11	.....	1175	52.50	9.64	Faint	.....	.....	.....	.....	.....	.....	.....	.....	"	"	"	.....
12	.....	563	39.40	7.82	Mod.	.....	76	53.8	86	Faint	13.3	0	13.3	"	"	"	0.5
13	.....	585	50.31	.....	Faint	.....	73	55.7	91	"	15.9	17.1	33.0	"	"	"	0.5
14	13.0	451	33.83	8.39	Doubtful	.....	.....	.....	.....	.....	.....	.....	.....	"	"	"	0.5
15	.....	575	51.46	9.06	Neg.	.....	.....	.....	.....	.....	.....	.....	.....	"	"	"	0.5
16	.....	310	11.25	7.09	Faint	.....	.....	.....	.....	.....	.....	.....	.....	"	"	"	0.5
17	.....	325	24.38	5.31	Neg.	.....	.....	.....	.....	.....	.....	.....	.....	"	"	"	0.5
18	.....	584	48.47	10.63	Faint	.....	.....	.....	.....	.....	.....	.....	.....	"	"	"	0.75
19	.....	936	63.84	13.74	"	.....	89	.....	75	Neg.	.....	.....	.....	"	"	"	0.75
20	.....	716	53.70	11.03	"	.....	.....	.....	.....	.....	.....	.....	.....	"	"	"	0.75
21	.....	652	49.65	9.58	"	71.7	159	.....	90	Faint	10.7	14.3	25.0	"	"	"	0.75
22	.....	374	28.80	6.81	Mod.	89.8	.....	.....	.....	.....	.....	.....	.....	"	"	"	0.75
23	.....	379	33.73	7.32	"	.....	.....	.....	.....	.....	.....	.....	.....	"	"	"	0.75
24	.....	575	33.35	9.55	Heavy	74.8	.....	.....	.....	.....	.....	.....	.....	"	"	"	0.75
25	.....	855	43.18	8.89	"	.....	77	51.9	98	Slight	.....	16.7	31.7	"	"	"	0.75
26	13.2	310	26.46	6.01	"	107.4	.....	.....	.....	.....	.....	.....	.....	"	"	"	0.75
27	.....	670	62.31	10.04	Mod.	.....	.....	.....	.....	.....	.....	.....	.....	"	"	"	0.75
28	.....	700	49.40	7.71	"	126.0	.....	.....	.....	.....	.....	.....	.....	"	"	"	0.75
29	.....	620	55.50	8.99	Heavy	148.8	.....	.....	.....	.....	.....	.....	.....	"	"	"	0.75
30	13.2	536	46.10	7.93	Mod.	117.9	.....	.....	.....	.....	.....	.....	.....	"	"	"	0.75
Oct. 1	.....	542	62.87	9.16	Faint	.....	.....	.....	.....	.....	.....	.....	.....	"	"	"	0.75
2	.....	945	63.32	7.09	Mod.	245.7	.....	.....	.....	.....	.....	.....	.....	"	"	"	0.75
3	.....	616	54.82	7.87	Slight	86.2	81	49.0	96	Neg.	.....	.....	.....	"	"	"	1
4	.....	632	58.78	9.80	"	176.9	.....	.....	.....	.....	.....	.....	.....	"	"	"	.....
5	.....	855	82.94	10.03	Mod.	.....	.....	.....	.....	.....	.....	.....	.....	"	"	"	.....
6	.....	605	50.22	8.65	Heavy	.....	.....	.....	.....	.....	.....	.....	.....	"	"	"	.....

TABLE 17  
Dog C3-98

Date 1916	Urine					Blood Plasma							Phlo- rizin gm.	Diet
	Wgt. kg.	Vol. cc.	Sugar gm.	Total N gm.	Nitro- prusside Reaction	Total Acetone gm.	Sugar mg. per 100 cc.	CO <sub>2</sub> Cap. Vol. %	Hgb. %	Nitro- prusside Reaction	Acetone and Diacetic Acid mg. per 100 cc.	B-oxy. (as acetone) mg. per 100 cc.		
Oct. 7	.....	710	68.58	9.93	Mod.	149.1	.....	.....	.....	.....	.....	.....	.....	400 gm. lung, 100 gm. suet and 15 gm. bread.
8	.....	890	66.75	9.26	"	.....	.....	.....	.....	.....	.....	.....	.....	"
9	13.1	622	55.36	8.58	Slight	68.4	94	51.9	85	Neg.	.....	.....	.....	"
10	13.1	680	60.86	7.07	Mod.	210.8	.....	.....	.....	.....	.....	.....	.....	"
11	.....	706	86.13	8.68	"	.....	.....	.....	.....	.....	.....	.....	.....	"
12	.....	638	56.78	6.76	Slight	.....	.....	.....	.....	.....	.....	.....	.....	"
13	.....	732	57.20	8.78	Faint	.....	.....	.....	.....	.....	.....	.....	.....	"
14	.....	630	54.35	7.56	"	.....	.....	.....	.....	.....	.....	.....	.....	"
15	.....	530	41.17	7.16	"	.....	.....	.....	.....	.....	.....	.....	.....	"
16	.....	654	58.21	9.81	Neg.	.....	.....	.....	.....	.....	.....	.....	.....	"
17	.....	640	57.09	11.14	Faint	.....	.....	.....	.....	.....	.....	.....	.....	"
18	.....	702	60.37	9.13	"	.....	.....	.....	.....	.....	.....	.....	.....	"
19	.....	684	52.67	10.60	"	.....	81	60.5	110	Neg.	.....	.....	.....	"
20	.....	628	55.89	11.01	"	.....	.....	.....	.....	.....	.....	.....	.....	"
21	.....	478	46.27	7.84	"	.....	.....	.....	.....	.....	.....	.....	.....	"
22	.....	700	70.07	12.18	"	.....	.....	.....	.....	.....	.....	.....	.....	"
23	.....	643	62.37	10.93	"	.....	112	60.5	95	Neg. Faint	.....	.....	.....	"
24	.....	725	60.18	7.32	"	.....	106	.....	.....	.....	.....	.....	.....	200
25	12.6	715	66.35	6.73	Mod.	66.4	.....	.....	.....	.....	.....	.....	.....	"
26	.....	650	60.22	8.78	Slight	71.5	.....	.....	.....	.....	.....	.....	.....	"
27	12.8	552	42.73	9.33	Faint	.....	.....	.....	.....	.....	.....	.....	.....	"
28	.....	220	Faint	7.52	Neg.	55.0	.....	.....	.....	.....	.....	.....	.....	"
29	.....	270	Neg.	9.69	"	40.5	.....	.....	.....	.....	.....	.....	.....	"
30	.....	202	"	5.70	"	18.2	.....	.....	.....	.....	.....	.....	.....	"
31	.....	286	"	9.95	"	.....	.....	.....	.....	.....	.....	.....	.....	"
Nov. 1	.....	88	"	.....	"	.....	.....	.....	.....	.....	.....	.....	.....	"
2	.....	280	Faint	9.01	"	.....	118	57.6	.....	Neg.	.....	.....	.....	"
3	.....	186	"	6.66	"	.....	.....	.....	.....	.....	.....	.....	.....	"
4	.....	190	"	7.41	"	.....	.....	.....	.....	.....	.....	.....	.....	"
5	.....	244	Neg.	8.47	"	.....	.....	.....	.....	.....	.....	.....	.....	"
6	.....	242	Faint	8.93	"	.....	.....	.....	.....	.....	.....	.....	.....	"
7	.....	240	"	10.80	"	.....	.....	.....	.....	.....	.....	.....	.....	"
8	12.5	318	"	7.47	"	Trace	120	57.6	82	Neg.	.....	.....	Trace	"
														and half pan bread and soup.

consistently explained by any known variables, such as the absolute or relative excretions of sugar or nitrogen, or the amount of body fat present. Dogs usually do not produce as much acetone as human beings, but some of them do excrete quantities which are fairly comparable per kilogram of weight with the output of some human diabetics.

3. Neither ketonuria nor ketonemia shows any parallelism with the curve of plasma bicarbonate. It is unfortunate that the circumstances did not permit trustworthy ammonia analyses, but we have mentioned elsewhere the impression that such dogs produce more ammonia than demanded for neutralization of their relatively scanty acetone bodies. It should be possible to determine experimentally whether unknown acids occur here in considerable quantities, or (if the supposition concerning ammonia proves correct) whether ammonia production has some other significance than neutralization of acids. Likewise if, as assumed, the power of dogs to excrete or neutralize acid is in excess of the demonstrable quantities of acid, the explanation of the terminal fall of plasma bicarbonate may lie between a hypothetical acidity of the tissues or an alteration of the distribution of bicarbonate between blood and tissues.

4. The intoxication and death in these animals are somehow connected with the state of ketosis, because they are prevented by anything (diet, renal impairment) that prevents the ketosis. There is, however, no known chemical basis of this intoxication. Death may come with either high or low acetone bodies in blood and urine, and with either high or low plasma bicarbonate. Neither alkali administration, nor liberal fluid administration (on account of dried tissues or diuresis to flush out poisons) is able to save life.

5. When a certain stage of intoxication is reached, carbohydrate administration may nearly or completely clear up the ketosis, but does not restore the normal plasma bicarbonate or save life. Any supposition that this fatal condition results from the previously high ketonemia or low blood alkali seems to be disproved by the fact that the same condition results when the ketonemia has never been very high or the plasma bicarbonate very low. It is interesting that this fatal terminal stage seems to be the same in all forms of ketosis, including the late stage of diabetic coma under insulin treatment.



6. An established ketonuria may not be immediately abolished by the feeding of protein or carbohydrate, perhaps because most of the available glucose is used first for glycogen formation. In this gradual clearing of ketonuria, the acetoacetic acid seems to disappear before the B-oxybutyric, and this fact may have some bearing on the question which of these two acids is formed primarily and which secondarily in metabolism.

7. The feeding of carbohydrate or protein, unless delayed to the fatal terminal stage, either prevents or greatly reduces the formation of acetone bodies, according to the quantities employed. The addition of fat has the opposite effect, but it is an interesting fact that ketosis is less with protein-fat diet than with fasting.

8. Some peculiar differences seem to prevail uniformly between phlorizinized and diabetic dogs with regard to acidosis. Fasting gives rise to the quickest and greatest acidosis in phlorizinized dogs, but fasting depancreatized dogs typically die in cachexia with little or no acidosis. Only long preparation and building up of weight by excessive fat diet brings partially depancreatized dogs to the point where they develop coma on fasting. These differences are perhaps explained essentially by the greater glycosuria of phlorizinized as compared with diabetic dogs in fasting. But with feeding the opposite difference is found. A liberal ration of protein and carbohydrate, along with fat, does not interfere with the production of fatal acidosis in a diabetic dog, while phlorizinized dogs with similar glycosuria seem to be able to endure such diets practically indefinitely with little, if any, acidosis and no serious impairment of health.

Two general conclusions are suggested. First, these observations are either incompatible with the theory that ketosis is determined solely by the ratio between carbohydrate and fat combustion, or they at least require explanation before the setting up of such a theory is justified. Second, this evidence agrees with the clinical facts regarding diabetic coma in indicating that the fatal disorder is not a mere intoxication with acetone bodies or a mere poisoning with acid, but rather an unknown metabolic derangement of which these chemical signs are only a superficial and variable expression.

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# GLUCOSE TOLERANCE AND ITS VALUE IN DIAGNOSIS

(Second Paper)

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## *Introduction*

### *Review of Literature*

It seems fitting to offer as a preface to this report of a second series of glucose tolerance estimations in 100 consecutive cases, a review of the literature pertaining to various phases of the problems presented by the interrelation of hyperglycemia and glycosuria.

The fact that most of the articles reviewed here have appeared since the preparation of the report of my first series<sup>26</sup> illustrates the rapid extension of interest in this direction.

In offering this brief summary, the difficulty of a proper analysis and correlation of the manifold data which have been published is manifest. In this, as in every other medical field, the importance of a uniform plan of accumulation and presentation of data should be emphasized.

This difficulty is well illustrated by the recent exhaustive survey of the literature on glucose tolerance by Horace Gray,<sup>19</sup> in which he emphasizes the importance in any study of glucose tolerance of "(1) a large series of normals more carefully chosen than has usually been the case; (2) agreement on the test substance and dose; (3) a minimum fasting interval, or a uniform time after a fixed preceding meal; (4) consistent use of whole blood (or plasma); (5) a constant chemical technique; (6) the same technician; (7) particular attention to psychic hyperglycemia by accepting with caution values obtained from inexperienced subjects or from the more painful punctures such as the finger tips."

While we are not in accord with all of Gray's conclusions, the very difficulties encountered by him in his attempt to correlate



this rich literature should serve as an incentive to further intensive study of these problems.

That the presence of sugar in the urine has some pathological significance and that it can be controlled by regulation of the diet appears to have been recognized at a very early date in medical history. In 1374 Bollaino, according to his own statement, was starved by his physicians because of sugar in his urine. In 1786, John Rollo was restricting the diet of patients with glycosuria. In 1851, Bouchardat inaugurated the observance of an occasional fast day as a means for controlling glycosuria. These are among the few names that appear in earlier literature in this connection.

The persistence of glycosuria, notwithstanding dietary control, is mentioned by various recent writers. Thus Allan<sup>1</sup> reports the case of a woman, 35 years of age, who had had glycosuria for 17 years. Her urine showed 4 per cent. of sugar, which persisted upon a very low diet. In spite of the high output of urinary sugar—16-49 gm. a day—the blood sugar was normal, the patient was in good health and presented no diabetic symptoms.

Bailey<sup>2</sup> reported a case of glycosuria which had been under observation for 10 years. Strouse<sup>44</sup> observed a case for eight years. Garrod<sup>14</sup> and Bonninger<sup>5</sup> each observed one for 6 years; Goto,<sup>16</sup> one for 5 years; Paullin,<sup>35</sup> one for 4 years, and Johnsson,<sup>28</sup> has recently reported two cases of a duration of 10 and 16 years, respectively.

In this connection Pollock's classification of different types of cases of glycosuria as given by Rainy<sup>37</sup> should be noted:

1. Cases in which the glycosuria is the result of renal action.
  - (a) Those without hyperglycemia (phlorizin);
  - (b) Those with hyperglycemia (renal poisons).
2. Cases in which the glycosuria is the result of hyperglycemia.
  - (a) Those in which it is independent of the glycogen content of organs, as is the case in true diabetes.
  - (b) Those in which it is dependent upon the glycogen content of organs and is caused by sympathetic stimulation, which may be either central or peripheral. Central stimulation producing glycosuria analogous to piqure is due to drugs, such as caffeine and strychnin, to asphyxia or to direct stimulation of the sympathetic sensory nerves. Peripheral stimulation producing glycosuria may be caused by adrenalin.

An editorial in the Journal of the American Medical Association<sup>6</sup> sums up so concisely our present conception regarding renal glycosuria that it seems well to quote it in full:

"The approach to a tenable conception of what is involved in the now commonly heard expression 'renal threshold for sugar' has come appreciably nearer through the demonstration that the so-called physiological glycosuria represents for the most part miscellaneous carbohydrates other than glucose, derived from the food and excreted more or less unchanged. Making due allowance for this more or less unidentified component of every urine, it now appears that in the absence of emotional complications or a subnormal renal threshold (renal glycosuria), the ingestion of pure glucose (up to 200 gm.) does not raise the level of the blood sugar above the threshold in normal persons and no glycosuria is obtained. In other words, there is no true 'glycuresis' in the sense in which this word has been defined by S. R. Benedict, after ingestion of large quantities of pure glucose by healthy man. Hyperglycemia does, of course, occur in such cases; but according to the evidence furnished by Folin and Berglund, hyperglycemia definitely below the threshold does not normally produce the slightest leakage of glucose through the kidneys and normally not a trace of absorbed and circulating glucose is lost. When glucose is excreted, it happens because the level of the blood sugar has risen above the normal threshold, or because the threshold itself is below normal, as in renal glycosuria.

"What is the physiologic mechanism by which the loss of glucose from the blood through the kidneys is ordinarily prevented? One hypothesis has referred it to glycogen formation averting excessive accumulation of sugars in the blood. Again, it has been assumed that ordinarily sugar is 'bound' in some way in the blood so that it is no longer 'free' or diffusible; elimination would then depend on the failure of the hypothetic sugar combinations in the organism. The latter view seems scarcely tenable in the light of the present day information regarding the condition of the sugar in both corpuscles and plasma. In contradistinction to such theories, Folin and Berglund have ventured the assumption that it is absorption by the tissues rather than glycogen formation that prevents excessive accumulation of sugars in the blood. They assume that the tissues always contain at least as high a concentration of free sugar as the blood plasma, and probably more. The glycogen formation need not begin until the tissues have begun to possess a much higher concentration than that present in fasting. Much absorbed sugar can thus be distributed without any large increases of the sugar in the blood.

"The role of the kidneys in relation to sugar is not ordinarily different from that of other tissues. Folin and Berglund argue that the strain comes only when the holding capacity of some tissues, including the kidneys, is exceeded. As a result of the strain thus produced, the kidneys are finally compelled to make use of a more efficient process than the glycogen formation for reducing the sugar concentration in the kidney cells and the elimination of sugar suddenly begins. If the attempt to direct attention more prominently to the avidity with which the tissues absorb sugar, like other products from the blood, becomes justified, the term alimentary glycosuria will become a misnomer."

The lack of dietary influence upon glycosuria in the presence of a low level of kidney permeability is well illustrated by the following references:

In an examination<sup>22</sup> of the urine of 159 persons, none of whom had had any apparent disturbance in carbohydrate metabolism, Holst found glycosuria in 19.5 per cent. after a meal, the amount of carbohydrates in which was smaller than that usually necessary to produce alimentary glycosuria.

Faber and Norgaard<sup>9</sup> report the case of a woman 29 years of age who for 3 years had been known to have renal glycosuria, but seemed to be in excellent health in spite of the fact that the urine contained from 60-80 gm. of sugar on an ordinary diet. In another case, a man 29 years of age had a history of glycosuria—1 per cent.—for 22 years; an older brother of this patient had a similar renal glycosuria, the sugar content of the blood in each being normal and the glycosuria persisting through the 24 hours. In a third case the urine contained only the normal amount of sugar in the morning, but after meals it ran up to 118-152, although the blood sugar kept within the normal range. It was thus a case of cyclic glycosuria of the renal type.

These authors tabulated the blood sugar findings in 32 diabetics at the moment when the urine was about free from sugar as the result of fasting.



The sugar content of the blood at this moment ranged from 190-90. In 16 of these patients this examination was repeated several times as glycosuria developed again, and again yielded to appropriate treatment, the intervals between these successive examinations ranging from one to twenty months. In every case the individual blood sugar threshold was found to be the same at each examination.

In a study of different methods for the determination of sugar excreted hourly after meals, Kast, Crale and Myers<sup>30</sup> found that the amount of sugar excreted on a diet rich in carbohydrates was greater than that on a low carbohydrate diet. With the exception of diabetes, in none of the diseases studied did the daily excretion of sugar in the urine show any marked variation from the normal excretion on ordinary diets. Moreover, in cases of diabetes, when by dietary regulation the patient was rendered "sugar free," the daily amount of sugar excreted was practically normal.

TABLE 1  
The Relation of Water Intake to Urine Output  
Non-Diabetic

No.	Intake	Output	% Inc.	% Decr.	No.	Intake	Output	% Inc.	% Decr.
1	1000	140	-----	86	26	950	470	-----	50.7
2	950	720	-----	24.2	27	900	595	-----	33.9
3	900	223	-----	75.1	28	860	1065	23.8	-----
4	920	960	4.3	-----	29	840	366	-----	56.3
5	850	1760	107.	-----	30	850	800	-----	5.8
6	1050	535	-----	96.5	31	850	320	-----	62.3
7	915	1140	24.6	-----	32	850	503	-----	40.7
8	4000	4305	7.6	-----	33	865	638	-----	26.2
9	850	670	-----	21.2	34	930	1010	8.44	-----
10	945	685	-----	27.5	35	850	855	5.9	-----
11	914	925	1.2	-----	36	930	361	-----	61.
12	980	730	-----	25.5	37	950	1038	9.2	-----
13	850	750	-----	11.8	38	850	605	-----	28.8
14	650	204	-----	68.5	39	850	830	-----	2.3
15	1140	1487	30.5	-----	40	950	310	-----	67.1
16	1010	215	-----	78.6	41	550	276	-----	50
17	725	630	-----	13.1	42	850	563	-----	33.7
18	800	1108	38.6	-----	43	880	235	-----	73.5
19	900	846	-----	5.4	44	850	201	-----	76.5
20	-----	-----	-----	-----	45	925	1305	41.2	-----
21	850	983	15.7	-----	46	850	728	-----	15.5
22	900	372	-----	56.5	47	950	1024	7.8	-----
23	885	538	-----	39.2	48	900	1115	23.9	-----
24	1000	566	-----	43.4					
25	910	590	-----	35.2					

Total number of cases 48.

Total number where the output is increased 15, percent 31.3  
" " " " " " decreased 23, " 68.7

In an investigation of 55 healthy subjects, Goto and Kuno<sup>17</sup> found that 33, or 60 per cent. eliminated sugar after having taken 100 grams of glucose, but that the quantity eliminated was very small; 28 of the 33 showed no abnormal hyperglycemia and in some cases the renal threshold for



glucose was lowered, the readiness with which sugar was eliminated in the urine after a carbohydrate diet being in inverse relation to the height of the kidney threshold. If the threshold was very low, sugar was excreted in the urine even when the sugar in the blood was at a normal level or slightly above the normal. Since the renal threshold for glucose is sometimes below the average, even in otherwise normal persons, these writers emphasize the importance of careful attention to the diagnosis of so-called mild diabetes in which, although slight glycosuria is present after meals, no clinical symptoms are noted. To make a differential diagnosis between mild diabetes and renal glycosuria it is necessary to examine not only the excretion of sugar in the urine but also the sugar in the blood after the glucose test has been made, and to study carefully the renal threshold for glucose.

The authors report that in this series the highest peak of the blood sugar curves was from 40-60 minutes after the ingestion of glucose, returning to the normal level within three hours. In 8 of the 14 subjects who excreted sugar in the urine, the glycosuria appeared at the following blood sugar concentrations: 122-129, 120, 122, 123-135, 139, 142-160, 146, 160 mg. per 100 cc. The renal function of those individuals, whose renal threshold for glucose was thus lowered, was normal for the excretion of water, urea and chlorides. The authors conclude that as the result of a lowered threshold for glucose glycosuria appears sometimes even in normal persons without any disturbance of carbohydrate metabolism and that therefore it is necessary to use great care in the differentiation of so-called mild diabetes and renal glycosuria.

Macleod<sup>32</sup> makes the statement that "although we cannot accurately measure the glucose excretion, it is very important to know whether the total carbohydrate in the urine of normal animals bears any quantitative relationship to the percentage of sugar in the blood." Using the charcoal method for removal of the interfering substances, Macleod, Christie and Donaldson found that from 1-1.85 grams of such material was excreted in the 24-hour urine of 2 normal men living on mixed diets containing a considerable proportion of carbohydrates. Using the mercuric nitrate method, Benedict, Osterberg and Neuwirth<sup>4</sup> found, in 2 normal men of 55 and 22 years of age, the total daily excretion to vary between 0.7 and 1.16 grams while living on ordinary diets, and to rise to a maximum of 1.6 grams when excess of carbohydrate was given. The ratio between the amount of fermentable and unfermentable sugar excreted also varied in relationship to the nature of the diet. The most significant results were secured by making the observation on urine voided every 2 hours during the day, the results being of the same nature on both of the subjects investigated, though quantitatively greater in the older man. After each of the three meals of the day, when these were rich in carbohydrates, an increase occurred in the fermentable sugar accompanied, at least in the forenoon, by a decided decrease in both the absolute and the relative amounts of the non-fermentable sugar. In the early morning (fasting) urine, the total reducing substance was small but the non-fermentable

variety relatively large. No definite relationship could be detected between the volume of the urine and the extent of the excretion of either fermentable or non-fermentable sugar. The increase in the fermentable sugar might be so marked as to bring its concentration up to the level at which the ordinary clinical tests for sugar were pronounced. The important practical conclusion is drawn from the results that "the normal organism is truly diabetic in that it has no absolute tolerance for carbohydrate." In other words, the difference between the normal and the diabetic is wholly a quantitative one.

Hammann and Hirschman<sup>21</sup> state that the normal renal threshold for glucose (0.17-0.18 per cent. of sugar in the blood) is found in many patients with lowered carbohydrate tolerance; and that some otherwise

TABLE II  
The Relation of Water Intake to Urine Output  
Diabetic

No.	Intake	Output	% Inc.	% Decr.	No.	Intake	Output	% Inc.	% Decr.
49	850	981	16	-----	76	850	340	-----	60
50	955	337	-----	65.7	77	920	885	-----	3.8
51	1175	1410	20	-----	78	710	700	-----	1.4
52	950	374	-----	60.6	79	900	517	-----	42.6
53	900	830	-----	7.7	80	830	667	-----	19.6
54	900	630	-----	30	81	845	271	-----	68
55	840	428	-----	49	82	845	1185	40	-----
56	950	150	-----	84	83	850	2110	148	-----
57	940	830	-----	11.7	84	850	700	-----	17.7
58	860	165	-----	81	85	950	570	-----	40
59	900	728	-----	19.5	86	1037	347	-----	66.8
60	850	610	-----	28.2	87	950	524	-----	44.8
61	950	483	-----	49.2	88	880	640	-----	27.4
62	850	1520	79	-----	89	900	688	-----	23.5
63	875	1150	31.4	-----	90	850	720	-----	15.3
64	850	1235	45.3	-----	91	1050	990	-----	57.2
65	840	735	-----	12.1	92	900	1000	11.1	-----
66	925	952	2.9	-----	93	925	470	-----	49.2
67	950	389	-----	59.1	94	920	700	-----	23.8
68	960	725	-----	24.5	95	950	570	-----	40
69	850	505	-----	40.7	96	950	525	-----	44.7
70	875	835	-----	4.5	97	830	575	-----	30.7
71	850	625	-----	26.4	98	950	805	-----	15.2
72	900	875	-----	8.3	99	980	615	-----	37.4
73	900	600	-----	33.3	100	900	420	-----	57.
74	850	1255	47.8	-----					
75	900	800	-----	11.1					

Total number of cases 52

Total number of cases where the output is increased, 10 - 19.2%

decreased, 42 - 80.8%

normal individuals have a low renal threshold (below 0.14 per cent.). They emphasize the importance of this observation in its bearing on our conception of "renal glycosuria." Many cases of nephritis have a high threshold, so that although the blood sugar may exceed 0.20 per cent. only a trace of sugar or none appears in the urine. Mild cases of diabetes

usually have a normal threshold, many severe cases have a lowered threshold; and this lowered threshold may be a factor in the severity of the disease. As the kidneys excrete sugar there is a tendency for the threshold to fall, so that although sugar may first appear when the blood sugar reaches 0.17 per cent., sugar may still be excreted after it has dropped to 0.10 per cent.

Adam Patrick<sup>45</sup> says that when the blood sugar concentration exceeds 0.18 per cent. the kidneys no longer hold it back and glycosuria develops. This level is known as the renal threshold for sugar. Glycosuria may occur in two different ways: by the rise of the blood sugar above the normal threshold, or by lowering the threshold below the blood sugar maximum. He thinks that the finding of glycosuria with a normal blood sugar curve is evidence of a lowering of the threshold, probably due to some peculiarity in the kidney.

Stefan Jörgensen and Tage Plum<sup>46</sup> traced the blood sugar curve in 17 non-diabetics, 15 diabetics, and 4 patients showing benign glycosuria. They found that with the intravenous injection of 20 gm. glucose, the highest percentage of blood sugar appeared directly after the injection. In normal subjects the blood sugar percentage fell very rapidly and the normal level was re-established in less than 90 minutes. In diabetics, however, the percentage of blood sugar fell rapidly at first, the curve of the fall resembling that of a normal person, but later on the curve tended to flatten out and become irregular. In none of the diabetic patients was the normal blood sugar level restored in 90 minutes, and in most cases more than two hours were required for the blood sugar level to reach the fasting state.

J. P. Bose<sup>47</sup> says that the value of these tests cannot be over-estimated. He has found that some types of curves could be associated with definite diseases. These types of curves, which were quite distinct from one another, were obtained for certain disorders of the ductless glands. Still more striking was the sorting out of a variety of kidney diseases, with abnormal amounts of sugar in the urine, but with normal and even sub-normal blood sugar. In hyperthyroidism, the blood sugar before the test was slightly raised (0.125%) according to the European standard, and likewise the urinary sugar (0.188%). The maximum rise was in 1 hour's time instead of  $\frac{1}{4}$  hour as in the normal individual; at the end of  $1\frac{1}{4}$  hours the blood sugar level was still high. There was an increase in the urinary sugar content after the test (0.568%). The noteworthy points about the curve are the steep rise, the maximum rise in 1 hour, and the high peak. In hyperpituitarism, the blood sugar before the test (0.082%), though normal, was associated with glycosuria. After giving 50 gm. glucose, the blood sugar showed a steady rise, which reached its maximum in 1 hour. There was a rise in the glycosuria after the test. The flattened top and the long-drawn-out blood sugar curve are considered to be typical of cases of this nature. In most cases of this type the glycosuria is intermittent. In hypopituitarism, the blood sugar before the test was raised, while the urinary sugar before the test was normal. The maximum rise



occurred in  $\frac{1}{2}$  hour, and, though the blood sugar increased considerably (0.58%, which is much above the threshold level), the urine did not contain any abnormal amount of sugar at the end of 1 and 2 hours. The curve shows the increased tolerance these patients have for carbohydrates. In renal glycosuria, though the blood sugar content before the test was quite normal, there was definite glycosuria. The maximum rise occurred in  $\frac{1}{2}$  hour, and within 1 hour the blood sugar fell to a very low level, rising and falling again in the course of the next hour. The curve shows that the test meal does not influence the blood sugar content very much.

G. W. McCaskey<sup>18</sup> says that the variability of the renal threshold in disease is very great, and even in apparent health variations are considerable, both in different individuals and in the same individual at different times.

He rightly thinks that the question of the renal glucose threshold deserves more clinical attention than it has received.

Geyelin,<sup>15</sup> while defining glycosuria accompanied by definite diabetic symptoms as being diabetic in type, goes on to say:

"There are other conditions, probably much more frequent than has hitherto been supposed, where the significance of glycosuria is much more problematic, namely when it is not accompanied by other evidence of diabetes. In these conditions the glycosuria is most often discovered accidentally in routine or life insurance examinations. Glycosuria may continue as a constant phenomenon or be intermittent in character and the health of the patient is very little, if at all, impaired. Many of these cases continue to show sugar in the urine for months or years, but if untreated, I think the majority eventually develop into outspoken cases of diabetes mellitus, or, as in other instances less frequently encountered, this glycosuria may continue for many years without the development of diabetes or even loss of weight. This latter form of glycosuria, provided that it meets with other diagnostic requirements which I shall speak of later, is termed 'renal glycosuria.' There is still a third form of mild glycosuria which is met with in certain toxic conditions, fevers, etc., but in these conditions the glycosuria is only transitory."

This author cites cases of renal glycosuria which had been under observation for several years, during which time they did not develop diabetes although they had been on a full diet.

George Graham<sup>18</sup> reported the case of a boy, 6 years old, who had been passing sugar in urine irregularly for the past 3 years, whose blood sugar rose from 0.11 to 0.16 per cent. in 30 minutes on 10 gm. of sugar; 0.12 per cent. in 60 minutes and 0.09 in 2 hours. This boy was on a restricted diet. The author stated that he was not sure as to the diagnosis but he was sure as to the treatment. He considered the raising of the "leak-point" in diabetics as a defensive mechanism.

Hamman<sup>20</sup> makes the following statement:—

"The glucose tolerance test, therefore, is a ready means to distinguish between renal and hyperglycemic glycosurias, when only a small amount of sugar is present in the urine. The character of the blood sugar response and the renal threshold are the deciding features. If the slight glycosuria be due to increased renal permeability, the condition may be dismissed as of little practical consequence. If on the other hand a definite meta-

TABLE III  
The Relation of Water Intake to Urine Output

	Series I		Series II	
	Diabetics	Nondiabetics	Diabetics	Nondiabetics
Total number of cases.....	57	43	52	48
Urine output greater than water intake.....	25.5%	42%	19.2%	31.3%
Urine output less than water intake.....	74.5%	58%	80.8%	68.7%

TABLE IV  
Highest Rise of Blood Sugar Content  
Non-Diabetic

No.	½ Hr.	1 Hr.	2 Hrs.	3 Hrs.	No.	½ Hr.	1 Hr.	2 Hrs.	3 Hrs.
1	109	-----	-----	-----	25	183	-----	-----	-----
2	100	-----	-----	-----	26	147	-----	-----	-----
3	-----	117	-----	-----	27	158	-----	-----	-----
4	-----	119	-----	-----	28	176	-----	-----	-----
5	108	-----	-----	-----	29	183	-----	-----	-----
6	128	-----	-----	-----	30	187	-----	-----	-----
7	-----	123	-----	-----	31	243	-----	-----	-----
8	130	-----	-----	-----	32	146	-----	-----	-----
9	145	-----	-----	-----	33	-----	159	-----	-----
10	133	-----	-----	-----	34	184	-----	-----	-----
11	164	-----	-----	-----	35	-----	174	-----	-----
12	-----	161	-----	-----	36	-----	153	-----	-----
13	135	-----	-----	-----	37	-----	194	-----	-----
14	143	-----	-----	-----	38	-----	171	-----	-----
15	139	-----	-----	-----	39	166	-----	-----	-----
16	-----	158	-----	-----	40	-----	189	-----	-----
17	146	-----	-----	-----	41	-----	-----	192	-----
18	143	-----	-----	-----	42	-----	-----	205	-----
19	163	-----	-----	-----	43	-----	286	-----	-----
20	184	-----	-----	-----	44	-----	230	-----	-----
21	150	-----	-----	-----	45	-----	-----	179	-----
22	158	-----	-----	-----	46	-----	280	-----	-----
23	-----	164	-----	-----	47	-----	-----	197	-----
24	170	-----	-----	-----	48	-----	238	-----	-----
Total Percent					48	28 58.1%	16 33.2%	4 8.3%	-----

bolic abnormality is discovered, then further study must decide whether the glycosuria indicates a mild diabetes, or is a symptom of some other disorder. There are a number of conditions besides diabetes in which a small amount of sugar in the urine, particularly occasional glycosuria, is observed. Chief among these are the disturbances of the thyroid and hypophyseal function, hypertension, nephritis and diseases of the brain. In acute nephritis, slight glycosuria occasionally occurs as a manifestation of impaired renal function with a lowered renal threshold for glucose. In essential hypertension and in chronic nephritis with hypertension, a little sugar is frequently found in the urine."

C. V. Bailey<sup>3</sup> says:

"The concentration of blood sugar at which glycuressis occurs varies in different individuals and is influenced by disease, being abnormally low in early diabetes, high in diabetes of long standing, in nephritis and in deficiency of the thyroid and hypophysis. Glycuressis is a kidney function and is excessive in diabetes and hyperthyroidism. It is greatly decreased in nephritis and in deficiency of the thyroid or hypophysis."

In a study of the blood sugar curves in a series of 200 cases, Olmstead and Gay<sup>34</sup> tried to demonstrate the main factors influencing the duration of hyperglycemia after a glucose meal and to establish a basis for the standardization of the technique of administration of the glucose meal. They reject the intravenous injection of glucose as impracticable. Janney's glucose meal of 1.75 gm. glucose per kg. body weight was used in their work. 60 to 80 per cent. of the ingested glucose was absorbed in the course of 2 hours. The curves they obtained indicated that for each individual the absorption rate is fairly constant. The majority of the curves reached the maximum height at the end of the first hour, with the second hour level usually lower. The pathological conditions in which the form of the blood sugar curve was usually constant were: hyper- and hypothyroidism, hypopituitarism, and diabetes mellitus.

Rathery<sup>38</sup> stresses the importance of the "indice glycémique de tolérance" as an element in prognosis, i. e., the sugar content of the venous blood on a diet comprising the extreme amount of carbohydrate that can be ingested without inducing glycosuria. This is based on the conception that each diabetic has his own individual glycemia tolerance index, and if this is high the prognosis should be reserved. When the carbohydrates ingested exceed the assimilable amount they seem to exert a directly toxic action.

Rosenberg<sup>39</sup> experimented on relations of the blood and of the urinary sugar to the amount of sugar consumed. In normal individuals, even when the blood sugar rose to 2 or 2½ times the normal, no sugar appeared in the urine; glycosuria appeared only after the ingestion of excessive amounts of grape sugar. When dextrose was introduced by rectum, thus avoiding the liver, glycosuria appeared with a slight hyperglycemia. The author states that it would appear that sugar, like albumin, by passing through the liver assumes a character adapted to the organism. As evidence of this noteworthy fact the author cites a case of intoxication by illuminating gas in which there was no glycosuria, although the blood sugar content was double the normal, while after the injection of grape sugar, though



there was a much lower hyperglycemia, sugar was excreted in the urine. When dextrose was given to normal individuals the blood sugar curve rose sharply, reached its highest point within half an hour or more and fell somewhat less abruptly; in diabetics it rose to a higher level but not so abruptly, reached its highest point sometimes in an hour and a half, but more frequently after 2 hours, the return to the normal level being in the form of an ellipse. The author states that by means of alimentary hyperglycemia a latent aglycosuric diabetes may be discovered and treated early, and therefore with better results than after a delayed diagnosis. In latent diabetes in elderly people, when the kidney with its diseased blood vessels does not react to the stimulus of the increased blood sugar, there may not be any glycosuria. This method can be used in differentiating between true and renal (innocent) diabetes, the latter showing a normal blood sugar curve in the presence of a pronounced glycosuria. The author states that it has not been proved, as some authors assert, that the presence of alimentary glycosuria can be relied upon for the early diagnosis of pregnancy. The condition varies in patients with Basedow's disease; some react normally to dextrose, while others show more or less pathologic variations from the diabetic curve. The author believes that by means of alimentary hyperglycemia it is possible to demonstrate pathologic changes of carbohydrate metabolism that cannot be found in any other way.

Holst<sup>23</sup> states that among the important practical results of the diabetic studies of late years is the fact that it has been possible to distinguish certain cases of glycosuria from genuine diabetes mellitus with greater certainty than formerly. Alimentary glycosuria may be produced in most cases by the ingestion of large quantities of sugar, the sugar concentration in the blood being thereby increased, the sugar entering into the urine when this exceeds the individual limit of assimilation. The quantity of sugar which can be ingested by healthy individuals without provoking glycosuria differs with the different kinds of sugar but almost all authors are agreed that glycosuria *ex amylo* is a sure sign of diabetes. As against this the author gives the following figures of A. T. Jacobsen and himself regarding the presence of glycosuria in non-diabetics after the ingestion of starchy foods: Jacobsen found glycosuria in 5 out of 24 normals or 21 per cent.; Holst, in 31 out of 159 normals or 19.5 per cent.

The author thinks that this alimentary glycosuria is due to a defective assimilation of carbohydrates. Such cases should not be treated as diabetes mellitus, but the patients should be advised to have their urine examined frequently and to abstain from eating foods containing much sugar.

Fernandez<sup>10</sup> emphasizes the following points: The presence of sugar in normal urine; the large proportion of sugar in the renal artery, the small proportion in the renal vein; the share of the kidney in the production of experimental phlorizin glycosuria; and the lesions in the kidney following glycosuria. He states that the glucose which reaches the kidney is partly destroyed and partly transformed into other bodies, only a small portion being eliminated; that glycosuria occurs when an excessive

TABLE V  
Highest Rise of Blood Sugar Content  
Diabetic

No.	½ Hr.	1 Hr.	2 Hrs.	3 Hrs.	4 Hrs.	No.	½ Hr.	1 Hr.	2 Hrs.	3 Hrs.	4 Hrs.
49	184	-----	-----	-----	-----	75	-----	-----	389	-----	-----
50	-----	254	-----	-----	-----	76	-----	-----	-----	-----	303
51	-----	-----	199	-----	-----	77	-----	-----	508	-----	-----
52	-----	191	-----	-----	-----	78	-----	-----	516	-----	-----
53	-----	248	-----	-----	-----	79	-----	-----	-----	405	-----
54	-----	-----	243	-----	-----	80	-----	-----	-----	537	-----
55	-----	250	-----	-----	-----	81	-----	-----	366	-----	-----
56	-----	-----	250	-----	-----	82	-----	-----	485	-----	-----
57	280	-----	-----	-----	-----	83	-----	-----	475	-----	-----
58	-----	300	-----	-----	-----	84	-----	-----	500	-----	-----
59	-----	252	-----	-----	-----	85	-----	428	-----	-----	-----
60	-----	-----	319	-----	-----	86	-----	-----	543	-----	-----
61	-----	-----	366	-----	-----	87	-----	-----	423	-----	-----
62	-----	-----	316	-----	-----	88	-----	-----	375	-----	-----
63	-----	-----	330	-----	-----	89	-----	-----	-----	575	-----
64	-----	-----	416	-----	-----	90	-----	-----	506	-----	-----
65	-----	-----	-----	467	-----	91	-----	-----	544	-----	-----
66	-----	-----	-----	460	-----	92	-----	-----	475	-----	-----
67	-----	-----	-----	460	-----	93	-----	428	-----	-----	-----
68	-----	-----	454	-----	-----	94	-----	-----	506	-----	-----
69	-----	-----	428	-----	-----	95	-----	-----	-----	300	-----
70	-----	-----	475	-----	-----	96	-----	-----	-----	294	-----
71	-----	-----	-----	454	-----	97	-----	-----	552	-----	-----
72	-----	-----	506	-----	-----	98	-----	-----	-----	434	-----
73	-----	-----	-----	475	-----	99	-----	-----	-----	-----	584
74	-----	-----	428	-----	-----	100	-----	-----	-----	832	-----
Total Percent						52	2 3.8%	8 15.4%	28 54%	12 23%	2 3.8%

TABLE VI

Comparison of Highest Rise of Blood Sugar Content after Ingestion of Glucose  
in Diabetics and Non-Diabetics

Highest Rise of Blood Sugar	Non-Diabetic Total Number of Cases, 48	Diabetic Total Number of Cases, 52
At end of ½ hour	In 58.0%	In 3.8%
At end of 1 hour	In 33.%	In 15.4%
At end of 2 hours	In 8.%	In 54.0%
At end of 3 hours	In 0	In 23.0%
At end of 4 hours	In 0	In 3.8%

amount of sugar is received, or the kidney function is insufficient; and that this final condition is liable to be present under various circumstances and must not be confounded with diabetes.

Savolin<sup>43</sup> discusses the glycosuria which appears when the thoracic duct is ligated or its contents diverted. This does not seem to bear any relation to diabetes mellitus and its cause is still a mystery. Savolin experimented on cats with pancreatic diabetes and analyzed some clinical cases in which the thoracic duct had been torn or compressed. In the latter cases no glycosuria developed; possibly collaterals maintained the circulation. He concludes that we have no grounds for assuming that the secretion of the pancreas reaches the blood by way of the thoracic duct.

Eliassow<sup>8</sup> gave 50 gm. of glucose and equi-caloric amounts of levulose, wheat flour and inulin to twelve sugar-free diabetics, and examined their blood and urine. Glucose increased the glycemia strongly, wheat flour less, levulose markedly less and inulin not at all. Eliassow believes that the slow absorption of inulin accounts for this.

Paullin and Sauls<sup>36</sup> have studied the relationship between diabetes and obesity. They selected 26 subjects who were from 10 to 80 per cent. overweight, in whom physical examination and laboratory tests showed as few abnormalities as possible which might influence their tolerance for sugar. Among these 26 persons, 9 of whom were between the ages of 30 and 50 years, 15 or 57.6 per cent. gave an abnormal response to the ingestion of glucose. Among these 15 the authors believe that at least 5, perhaps 6, were in the early stages of diabetes. These individuals exhibited no clinical evidence of the disease, and it is possible the other 9 cases may follow a similar course unless the dietary regimen instituted for them is carefully followed. Of the 15 who responded abnormally to glucose, all were over 20 years of age and at least 30 per cent. overweight. Although the number of cases studied was small, the authors feel that their findings are significant and that by this study a prediabetic condition was discovered in at least 5 patients, two of whom have definitely developed diabetes. As the authors say, the test is simple, reliable and of value in stressing the importance of dietary therapy in these cases.

In two previous articles<sup>24-25</sup> of my own I have reported cases of a low renal permeability in normal individuals. One, an 18 year old girl, had been treated as a diabetic for several months and nearly starved to death, her weight falling from 123 to 63 pounds. Since glucose tolerance estimations have shown that this was a case of simple glycosuria the girl has been on a full diet and has shown no signs of a hyperglycemia.

In my first series<sup>26</sup> of 100 cases in which glucose tolerance estimations were made in 27 non-diabetics and 5 diabetics who showed glycosuria, the blood sugar was 125 mg. per 100 cc. or less, and in a series of 714 blood sugar estimations in 99 cases which showed no glycosuria, the blood sugar was 120 mg. per 100 cc. or less.

Fitz, Beeler and Bryan<sup>11</sup> advocate aspirating the stomach one hour after the ingestion of glucose, subtracting the glucose recovered from the



intake and calculating on the basis of what has been absorbed per kg. body weight. They recovered from 7.7 to 16.6 per cent. of glucose in their first series and from 48 to 65.5 per cent. glucose in their second series of cases. This paper is of scientific importance but the procedure is not practicable from our point of view.

The relation of glycosuria and of hyperglycemia to other pathological conditions than diabetes is discussed by various writers.

San Marten<sup>42</sup> calls attention to the variations which accompany hyper- or hypo-function of the thyroid gland. He states that hyperthyroidism is frequently accompanied by hyperglycemia and even glycosuria, while in hypothyroidism, myxedema and cretinism, the tolerance for carbohydrates is greatly increased. Following extirpation of the thyroid gland, the parathyroids being left intact, glycosuria never occurs even after the ingestion of large quantities of sugar. The author cites the case of a woman 29 years of age who had never been well, was of slight stature, weighed only 90 pounds, and whose menstruation had always been irregular and had ceased at the age of 18. She had had diabetes for 4 years, this yielding to treatment by the Allen method. She presented Basedowian symptoms consisting in an increase in the volume of the thyroid gland, exophthalmos, tachycardia, suffocation, gastric disturbances, hyperacidity and nervous instability. There was a persistently low tolerance for carbohydrate, in spite of treatment. A severe pain in the right iliac fossa demanded operation, and an exploratory laparotomy was performed. The right ovary was found to be sclerotic and this and the appendix were extirpated. The genital organs were infantile. The post-operative course was favorable except for an acute delirium of several days' duration. There was no recurrence of pain, the tolerance for fat and protein was high, but carbohydrate readily caused glycosuria. The author cites briefly three parallel cases. He concludes that hyperthyroidism plays a fundamental etiologic role in the disturbance of endocrine equilibrium which constitutes the diabetic syndrome.

Johnsson<sup>29</sup> reports 2 cases of a man and a woman, the diagnosis in the first case being gastric ulcer, latent syphilis and renal glycosuria, in the second, tape-worm, uterine myoma and renal glycosuria. In the first case the quantity of the urinary sugar remained practically unchanged for 10 years and in the second for 16 years. In both cases, the carbohydrate test feeding was followed by normal or diminished blood sugar content. The usual symptoms of diabetes were lacking in both cases. Neither patient was robust, and both had digestive disturbances. Gastric ulcer was demonstrated in the first case and it is reasonable to believe that it might have been demonstrated in the second. There was a diabetic or glycosuric family history in the second case.

In a study of 140 cases of diabetes mellitus Rosenbloom<sup>41</sup> found a positive Wassermann reaction in 16 cases—11.4 per cent. Eight of these presented signs of tertiary syphilis. No increase in tolerance for carbohy-

TABLE VII  
The Renal Threshold.

Blood Sugar.....	40	50	60	70	80	90	100	110	120	130	140	150	160	170	180	190	200	210	220	230	240	250	300	350 and up
Mgm/100 cc.....	50	60	70	80	90	100	110	120	130	140	150	160	170	180	190	200	210	220	230	240	250	300	350	
Non-diabetic With glycosuria.....	1	.....	5	7	4	4	3	3	3	3	2	3	1	2	2	2	3	.....	.....	1	.....	2	.....	.....
Without glycosuria.....	3	4	14	27	23	35	21	11	8	11	3	5	2	3	.....	2	.....	.....	.....	.....	.....	.....	.....	.....
Diabetic With glycosuria.....	.....	.....	.....	.....	.....	.....	.....	.....	6	3	1	2	5	6	2	3	5	2	1	4	9	30	34	101
Without glycosuria.....	.....	.....	1	1	2	4	5	4	6	3	4	3	5	1	.....	3	3	1	3	2	.....	.....	.....	.....

Total number of observations: 508.

drates followed treatment. The author believes that this may be due to the fact that the fibrosis of the pancreas produced by the syphilis is not changed by the treatment.

Lemann<sup>31</sup> has made a study based on his experience at the Charity Hospital in New Orleans, where among the 61,298 admissions from 1898 to 1909 he found that 45 white patients (.073 per cent.) and 19 negroes (.03 per cent.) had diabetes. The negroes furnished 40 per cent. of the admissions, yet among them were included only 30 per cent. of the diabetics. From 1910 to 1919 inclusive there were 160,044 admissions, among which there were 135 white patients with diabetes, or .084 per cent. and 59 negro diabetics, or .036 per cent. Here also the negroes furnished 43 per cent. of the admissions but only 30 per cent. of the diabetics. During the same period more than 50 per cent. of all the syphilitic diseases were in negroes. From these figures the author concludes that there is no relation between the incidence of diabetes mellitus and syphilis and that therefore there is no probable etiological relation between the two. Moreover, there is an unexplained immunity in the negro race to the production of the spirochetal pancreatitis just as there is an unexplained immunity in that race to the production of locomotor ataxia.

Among 139 cases of diabetes mellitus Rosenbloom<sup>40</sup> found 16—over 12 per cent.—in which the Wassermann test was positive. Eight of these presented signs of arteriosclerosis. It was possible that these 8 cases of diabetes were of a syphilitic nature and referable to the arteriosclerosis. There would undoubtedly be present some fibrosis of the pancreas. These 8 cases were subjected to intensive treatment for the syphilis, but there was no increase in their tolerance for carbohydrate. This is no doubt due to the fact that the pancreatic fibrosis still existed after the treatment. He concludes that in about 6 per cent. of the cases studied diabetes existed as a part of the syphilitic process, and in about 6 per cent. the syphilis and diabetes existed as independent conditions.

## PROCEDURE

This was the same as in our previous report. The first blood specimen in each case was taken in the morning before any food had been eaten, after which glucose was given as described below and no other food was permitted until the completion of the test. During the period of four or more hours required for the complete study, each patient remained in a private room where he was at liberty to sit up and read or to lie down as he chose.

Whenever it was possible, the patient was advised not to urinate in the morning until his arrival at the Clinic, in order that the urine might be secured just before the test. In any case the bladder was emptied before the first test was made. This accounts for the large urine output at the beginning of some of the studies. This amount, however, is not included in the calculation of the total output of urine during the period



covered by the study, as this always started with an empty bladder. This initial specimen was used only as a control, to see whether or not the patient "started" with a glycosuria.

The first specimen of blood amounts to about 12-18 cc. in order to have a sufficient quantity for the determination of chlorides, urea, uric acid, creatinin, and for the Wassermann reaction, etc., in addition to the blood sugar estimation. From 6 to 8 cc. is taken for each of the subsequent tests.

Following the taking of the first specimen, the patient is given 100 gm. of anhydrous glucose dissolved in 250-350 cc. of water, to which the juice of one or two lemons is added. This solution is less nauseating if it is ice cold. The time is noted, and specimens of blood are taken at the end of the following periods—one-half hour, one hour, two, three and four hours. At the end of each hour the patient voids, each time in a separate jar, and is given 200 cc. of cold water to drink. The last water taken is not included in the total water intake, however, as no later specimen of blood is taken.

On each sample of blood the following observations are made: 1) sugar content of whole blood; 2) sugar content of plasma; 3) sugar content of corpuscles; 4) corpuscle volume; 5) a series of estimations of the sugar content by the Epstein method.

On each specimen of urine the following observations are made: 1) total volume; 2) specific gravity; 3) presence or absence of sugar, qualitatively determined by the Benedict reagent; 4) sugar content, quantitatively estimated by the Benedict method.

The blood sugar estimation is made by Myer's modification of Benedict's method, using the Kober colorimeter. The estimation of the corpuscle volume is made by centrifuging the oxalated blood at the rate of 3000 revolutions per minute for 10 minutes.

*Explanation of charts.*—The checkered columns at the top of the charts indicate the water intake; the solid black columns at the top indicate the urine output. The total intake and output during the period of four hours or more is indicated by a like marking at the lower right corner of the chart. The circles at the lower ends of the black columns indicate the presence of sugar in the urine.

The broken horizontal line opposite "120" is the upper limit of normal blood sugar, i. e., 120 mg. per 100 cc. of blood. The broken vertical line opposite "3 hours" is the period within which, in normal individuals, the blood sugar content again becomes normal after the ingestion of the standard dose of glucose. The heavy curve represents the blood sugar content at the designated periods. The dots which break the glucose tolerance curve indicate the intervals at which blood was taken for sugar estimation. Each solid black column at the bottom of the charts represents the sugar content of the urine output indicated by the corresponding solid black column at the top of the chart. Each square included in these lower columns represents one gram of sugar, and the total sugar

output—the sum of these squares—is indicated by the solid black portion of the large square at the right of the chart, which includes 100 squares, representing 100 grams of glucose—the total sugar intake.

An anamnesical summary of each of the 100 cases is given in Table XX and this should be consulted in the study of the charts of the individual case, in order that each blood sugar curve may be properly related to age, sex, occupation, familial history of diabetes, previous infection, etc.

TABLE VIII  
Renal Threshold.

mg/100 cc.....	50 75	75 100	100 125	125 150	150 175	175 200	200 225	225 250	250 300	300 up
Non-Diabetics.....	7	6	2	2	1	.....	1	.....	....	.....
Diabetics.....	....	.....	.....	9	5	3	4	5	13	14

TABLE IX  
Percentage of Excretion of Sugar at Different Hour Periods.  
Non-Diabetic.

No.	Total Hrs.	Gms. Total Sugar	Gms. 1 Hr.	%	Gms. 2 Hrs.	%	Gms. 3 Hrs.	%	Gms. 4 Hrs.	%	Gms. 5 Hrs.	%
4	2	.234	.112	48	.122	52	.....	.....	.....	.....	.....	.....
16	2	.37	.373	100	tr.	.....	.....	.....	.....	.....	.....	.....
21	3	.598	.195	32.6	.403	67.4	.....	.....	.....	.....	.....	.....
24	1	.39	.39	100	.....	.....	.....	.....	.....	.....	.....	.....
26	3	3.84	1.48	38.5	1.44	37.5	.91	24.	.....	.....	.....	.....
28	2	.6	.46	76.5	.14	23.5	.....	.....	.....	.....	.....	.....
31	2	.6	.....	.....	.6	100	.....	.....	.....	.....	.....	.....
33	2	2.9	1.	34.5	1.9	65.5	.....	.....	.....	.....	.....	.....
34	3	1.85	.....	.....	1.1	59.4	.75	40.6	.....	.....	.....	.....
37	2	.741	.09	12.6	.64	87.4	.....	.....	.....	.....	.....	.....
40	2	.302	.248	82	.054	18	.....	.....	.....	.....	.....	.....
42	2	.18	.....	.....	.18	100	.....	.....	.....	.....	.....	.....
43	3	1.158	.236	20.5	.715	61.8	.207	17.7	.....	.....	.....	.....
44	3	0.799	.275	34.6	.281	35.2	.243	30.2	.....	.....	.....	.....
46	2	1.156	.558	48.4	.598	51.6	.....	.....	.....	.....	.....	.....
47	4	2.	.079	3.95	1.407	70	.299	14.9	.215	11.2	.....	.....

As in the preceding series, a graphic chart of each case is given in the belief that only in this way can individual characteristics be displayed and the exact reactions of a sufficient number of normal and abnormal individuals be shown to gain evidence for the final formulation of principles which can safely govern our later judgments and procedures. The broader results of the investigation are grouped and summarized in a series of general tables and charts which are discussed under various headings.

## DISCUSSION OF FINDINGS

*Relation of Water Intake to Urine Output.* The relation of the water intake to the urine output is given for each case in Tables I and II, p. 258 and p. 260, and shown graphically in Charts I and II, p. 297. A summary of these findings, as compared with the findings in the preceding series, is given in Table III, p. 263, which shows that in each series the urine output is distinctly less in the diabetic cases than the water intake, this difference being present also, but less marked, in the non-diabetics. Following the ingestion of glucose, there is a definite retention of water in the circulation and probably also in the tissues, the successive stages in this retention following in a general way the reduction of the hyperglycemia back to the normal or subnormal blood sugar content. This water retention is more marked in the diabetic cases because of the longer persistence of the hyperglycemia, although as seen by the tables and charts there are also diabetic cases in which the urine output is greater than the water intake. It is interesting to note that in some cases the largest output of urine coincides with the peak of the blood sugar curve, as for instance, in case 62, at the end of the 2 hour period. I am unable to explain this occurrence, for it is contrary to what one would expect. In case 63, the output increases after the blood sugar curve begins to descend, being greatest at the end of the 4 hour period.

In case 64, the largest output is at the end of the third hour, when the blood sugar curve has descended but little from its highest peak. In case 74, there is an increased output of urine at the end of the second hour, the largest amount being at the end of the third hour, which marks the peak of the blood sugar curve. Case 82 starts with an increased urine output at the end of the first hour, when the blood sugar curve is on the ascent, the increase continuing during the second and third hours. A like variation is especially marked in case 83, in which a very large urinary output starts at the end of the first hour, when the blood sugar curve is on the ascent, with the most profuse output at the end of the third hour when the curve begins to descend. One might surmise that possibly in these cases the



diabetes was of recent development and that therefore the diabetic equilibrium had not become established as in the cases of longer standing; but this is not so, for only about one-half of these cases were of recent development, the other half being cases of some years' standing. There must, therefore, be some individual characteristic which is peculiar to these atypical cases, but what it may be I am unable to suggest.

TABLE X  
Percentage of Excretion of Sugar at Different Hour Periods.  
Diabetic.

No.	Total Hrs.	Gms. Total Sugar	Gms. 1 Hr.	%	Gms. 2 Hrs.	%	Gms. 3 Hrs.	%	Gms. 4 Hrs.	%	Gms. 5 Hrs.	%
48.....	2	.31			.31	100						
49.....	3	.647	.244	38.6	.2	30.7	.2	30.7				
52.....	2	.13			.13	100						
53.....	3	.863	.144	19.	.254	29.4	.495	31.6				
54.....	3	1.258	.088	7.05	1.04	80.0	.124	13.				
55.....	3	1.	.13	13.	.64	64.	.23	23.				
56.....	4	2.82	.22	7.8	1.12	39.6	.88	31.2	.6	21.4		
57.....	4	8.94	.735	8.24	2.765	31.	4.29	48	1.15	32.8		
58.....	4	4.23	.59	73.9	1.47	34.7	1.62	38.2	.03	3.2		
59.....	3	2.59	.163	6.3	1.36	52.3	1.07	41.4				
60.....	3	2.9	.36	12.4	2.04	70.	0.5	17.6				
61.....	4	6.12	1.04	16.5	2.99	48.8	1.89	30.8	0.2	3.9		
62.....	4	13.15	.45	3.38	4.	30.	6.11	46.	2.58	20.62		
63.....	4	3.17	.26	8.2	1.61	50.6	1.26	39.8	.04	1.4		
64.....	4	8.63	.9	12.2	3.36	38.8	3.24	37.6	1.13	11.4		
65.....	4	12.01	.55	4.57	2.09	17.4	4.5	37.4	4.87	40.7		
66.....	4	16.78	1.	5.99	4.2	25.	6.1	36.4	5.39	32.7		
67.....	4	7.82	.67	8.55	2.28	29.3	3.77	48.2	1.1	14		
68.....	4	10.36	2.34	22.	4.65	45.8	1.57	15.1	1.8	17.1		
69.....	4	10.73	.91	5.25	6.1	56.8	3.41	31.8	.31	6.2		
70.....	4	14.7	2.09	14.9	3.42	22.9	5.77	39.3	3.42	22.9		
71.....	4	2.92			.38	13.	1.53	52.5	1.01	34.5		
72.....	4	6.73	.36	5.35	1.82	27.	2.98	44.2	1.57	37.5		
73.....	4	12.95	.21	16.2	3.1	23.9	4.56	35.5	3.08	24.4		
74.....	4	12.81	.31	2.42	3.09	23.5	6.	47.4	3.41	26.7		
75.....	4	4.91	.19	3.86	1.87	38.1	1.63	33.2	1.22	24.9		
76.....	4	9.21	.05	5.42	1.75	19	3.5	37.7	3.91	37.9		
77.....	4	31.86	5.4	18.6	10.44	31.6	10.8	31.8	5.22	18.0		
78.....	3	8.83	1.32	14.9	3.15	42.	3.36	43				
80.....	4	18.77	1.2	6.42	4.4	23.4	8.1	43.2	4.9	27		
81.....	4	8.98	.98	10.9	2.36	26.2	2.8	31.2	2.84	31.7		
82.....	4	18.17	3.35	18.5	5.58	30.7	6.2	34.2	3.04	16.6		
83.....	4	23.17	3.08	13	9.55	41	7.35	31	3.19	15		
84.....	4	8.95	.816	9.	2.34	26.	3.	33.	2.8	32.		
85.....	4	11.12	1.84	16.5	3.76	34.	3.72	34.	1.8	15.5		
86.....	4	7.71	.73	9.4	3.87	50	2.56	33	.55	7.6		
87.....	4	8.32	.59	7.	3.71	44	2.83	34	1.19	15		
88.....	4	18.64	3.12	16.	5.16	27.	7.98	42	2.38	15		
89.....	4	15.87	3.92	25.	4.27	27.	4.18	26	3.5	22		
90.....	4	14.18	3.19	22	4.42	31.	4.5	31	2.07	16		
91.....	4	34.34	7.35	21.	14.50	42.	9.3	27	3.19	10		
92.....	4	24.64	5.4	22.	6.44	26.	8.75	35	4.05	17		
93.....	4	10.04	0.9	9	3.	30.	3.48	35	2.66	26		
94.....	4	16.29	1.56	9	5.64	37.	5.52	37	3.57	17		
95.....	4	8.74	0.1	11.	2.1	23.	3.67	42	2.87	24		
96.....	4	7.37			1.12	15.	3.	40	3.25	45		
97.....	4	15.86	2.25	14	3.15	20.	6.3	39	4.16	27		
98.....	4	18.22	2.2	13	2.7	15.	6.66	36	6.666	36		
99.....	4	20.81	1.87	9	4.78	23.	5.8	28	8.36	40		
100.....	4	17.81			4.86	28.	5.2	29	7.75	43		

*Period Within Which the Highest Rise of Blood Sugar Content Occurs.* In general in the non-diabetic, the blood sugar content rises promptly after the ingestion of glucose and there is an equally prompt return to the normal level or below, whereas in the diabetic the blood sugar curve rises slowly and to a higher level with a correspondingly slow return of the curve to the normal level. This comparison is well shown by Tables IV and V, p. 263 and p. 266, and is graphically illustrated by Chart III, p. 298; Table VI, p. 266, summarizes and compares the findings in this with the preceding series. In the diabetic, the return to the normal level takes from 4 to 9 hours, whereas in the non-diabetic this return to normal is accomplished in from 1 to 2 hours. Thus, in the diabetic individual, the blood is flooded with sugar for many hours, whereas in the non-diabetic it is so flooded for but a short period.

The above represent typical cases in each group, but there is a large group in whom the blood sugar picture is not so clearly defined, and it is necessary to decide within what limits a blood sugar curve may be considered as normal. It is generally conceded that any case in which, following the ingestion of 100 gm. glucose (or its equivalent in the case of children), the blood sugar curve returns to the normal level in 3 hours, is considered as normal; and one in which the blood sugar curve returns to the normal level after more than 3 hours is classified as diabetic. Naturally there is not much difference between a patient whose blood sugar curve returns to the normal level in 2 hours and 45 minutes and one whose curve returns to the normal level in 3 hours and 15 minutes. Such cases form a distinct group by themselves and we consider them as having a mild form of diabetes or as being in a prediabetic stage. It is this group which presents the most serious challenge to the physician, from the standpoint both of treatment and of prevention. It is well known that in a case of fully developed diabetes but little can be accomplished by treatment, in a fundamental sense, apart from symptomatic improvement. Only in the early cases can we accomplish the desired result, i. e., increase the tolerance for carbohydrates. These prediabetic, or mild cases, can be maintained in the prediabetic stage and kept from becoming fully developed diabetics. The blood sugar of such cases can be kept normal by means of only a slight reduction of diet and they can be maintained in

good health with their economic status unimpaired. Once diabetes has become fully developed, this can no longer be accomplished by diet, and recourse must be had to insulin.

*Has the height of the blood sugar curve any significance?* Our findings in this series again correspond with those of the preceding series. In some normal individuals, as stated above, the blood sugar curve rises to a high level with an equally abrupt fall. Apparently as the result of some individual, but insignificant factor, the blood is for the moment, as it were, overwhelmed

TABLE XI

Total Excretion of Sugar.  
(Intake 100 gms.)  
Non-Diabetic.

No.	Hours	Sugar gms.	Percent Excreted	No.	Hours	Sugar gms.	Percent Excreted
4	2	.234	.2	34	3	1.85	1.8
16	2	.37	.3	37	2	.741	.7
21	3	.598	.5	40	2	.302	.3
24	1	.39	.3	42	2	.18	.1
26	3	3.84	3.8	43	3	1.158	1.1
28	2	.6	.6	44	3	.799	.7
31	2	.6	.6	46	2	1.156	1.1
33	2	2.9	2.9	47	4	2.	2.

by the sudden entrance of a large quantity of sugar into the circulation and is unable to handle it as promptly as in other cases; but soon the regulatory mechanism is stimulated and adjustment takes place. It is the time period covered by the total curve of the rise and fall of the blood sugar, which after all is the significant criterion.

*The importance of the blood sugar content at the end of the first half-hour after the ingestion of glucose:* In the previous series, the highest rise of blood sugar appeared one-half hour after the ingestion of glucose in 50.8 per cent. of the non-diabetic cases and in only 4.6 per cent. of the diabetic. The corresponding figures in this series are 58.1 per cent. of the non-diabetic cases and 3.8 per cent. of the diabetic. This comparison, of course, is



of academic interest only as it conveys no practical information, the sugar content of the fasting and of the three-hour specimens being the basis for the decision as to the diabetic or non-diabetic status of the individual.

*Is a delayed rise of blood sugar due to lack of absorption from the intestine?* Friedenwald and Grove<sup>13</sup> show the blood sugar curves observed by them in cases of carcinoma of the gastro-intestinal tract and state their belief that the glucose tolerance test may be utilized as a method of differentiating between malignant and benign conditions, "and that while it cannot in any way be considered specific for carcinoma, when taken into consideration with the other clinical evidence, it may serve as a valuable aid in diagnosis in obscure cases of carcinoma of the gastro-intestinal tract." It is true that the curve shown by them indicates a slow absorption of glucose from the intestinal tract, but it is a typical diabetic curve. Diabetes results from anatomic or functional damage to the islands of Langerhans, and two of the cases included by these authors are stated to have been cases of carcinoma of the pancreas. A delayed rise of the blood sugar curve may be due, it is true, to lack of absorption from the intestine, but if the individual is non-diabetic this rise and its plateau will not persist, and the sugar will promptly be taken care of, whereas only in a diabetic individual will the hyperglycemia persist. These authors say, "It is important to note that diabetes, nephritis, tuberculosis and disturbances of the thyroid should always be excluded before the tolerance test is undertaken, inasmuch as hyperglycemia is frequently present in these affections." It is a well known fact that diseases and infections of various kinds—measles, mumps, scarlet fever, influenza—may cause diabetes. Why exclude them? Diabetes is diabetes, regardless of its cause; and it is identified by fasting hyperglycemia and by characteristic findings from a glucose tolerance estimation. If the diabetes remains untreated, the patient may die from it, whatever other condition may be associated.

*The renal threshold.* As we have stated repeatedly and as is manifest from the literature, the renal permeability to sugar varies widely in individual cases. Individuals with the most severe type of diabetes may show no sugar in the urine and normal individuals may have a persistent glycosuria. In every

individual the kidneys become permeable to sugar at some blood sugar level; but this level varies in different normal individuals, in different diabetics and no doubt in the same individual at different ages, although as to the last point we have not sufficient observations on single normal individuals over a period of many years to justify any conclusion. The important point is that in a diabetic case the permeability varies widely in direct

TABLE XII  
Total Excretion of Sugar.  
(Intake 100 gms.)  
Diabetic.

No.	Hours	Sugar gm.	Percent Excreted	No.	Hours	Sugar gm.	Percent Excreted
48	2	.31	.3	75	4	4.91	4.9
49	3	.647	.6	76	4	9.21	9.2
52	2	.13	.1	77	4	31.86	31.8
53	3	.063	.8	78	3	8.83	8.8
54	3	1.258	1.2	80	4	18.77	18.7
55	3	1.	1.	81	4	8.98	8.9
56	4	2.82	2.8	82	4	18.17	18.1
57	4	8.94	8.9	83	4	23.17	23.1
58	4	4.23	4.2	84	4	8.95	8.9
59	3	2.59	2.5	85	4	11.12	11.1
60	3	2.9	2.9	86	4	7.71	7.7
61	4	6.12	6.1	87	4	8.32	8.3
62	4	13.15	13.1	88	4	18.64	18.6
63	4	3.94	3.9	89	4	15.87	15.8
64	4	8.63	8.6	90	4	14.18	14.1
65	4	12.01	12.	91	4	34.34	34.3
66	4	16.78	16.7	92	4	24.64	24.6
67	4	7.82	7.8	93	4	10.04	10.
68	4	10.36	10.3	94	4	16.29	16.2
69	4	10.73	10.7	95	4	8.74	8.7
70	4	14.7	14.7	96	4	7.37	7.3
71	4	2.92	2.9	97	4	15.86	15.8
72	4	6.73	6.7	98	4	18.22	18.2
73	4	10.95	10.9	99	4	20.81	20.8
74	4	12.81	12.8	100	4	17.81	17.8

relation to the length of time that hyperglycemia persists, so that if in an early case of diabetes the kidneys are permeable at a blood sugar concentration of 160 mg. per 100 cc., a few years later, if the patient is untreated, the permeability threshold may be somewhere near 200-250 mg. per 100 cc. As to whether this permeability threshold remains unchanged in diabetics whose blood sugar concentration is kept at a normal level by treatment, we still lack observations.

Tables VII to XIII and Charts IV to VIII, p. 298 and p. 299, show the relationship of the renal threshold to the blood sugar concentration. Table VIII, p. 272, shows that among 19 non-diabetics the threshold in 17 (90 per cent.) was below 150 mg. per 100cc., while among 53 diabetics the threshold was below 150 mg. per 100 cc. in only 9 cases (17 per cent.) and above in 44 cases (83 per cent.); that is, among the diabetics the permeability level is greatly raised. However, there are marked exceptions to this generalization. For example, I have a little diabetic patient, a girl four years old, not included in this series, whose permeability level is below 80 mg. per 100 cc. From this series it would appear that were we to accept the formerly accepted standards of urinary analysis as the criterion for the diagnosis of diabetes, we

TABLE XIII

Renal Threshold.

	1st Series of 100		2nd Series of 100	
	170 mg/100 cc. or below	Above 170 mg. per 100 cc.	170 mg/100 cc. or below	Above 170 mg. per 100 cc.
Non-diabetics.....	34	1	18	1
Diabetics.....	12	12	14	39

should have treated as diabetics 15 of the 19 cases included in Table VIII as non-diabetics, and we should have missed all the diabetics who by chance diet had kept their blood sugar a bit below the permeability level. This is a common occurrence, for in the milder diabetic cases, especially in the elderly, it often takes only a moderate reduction in diet to bring the blood sugar below the permeability level. The term "sugar free" in a diabetic, therefore, means very little unless one knows the permeability level of the individual in question. Not urinary sugar but blood sugar must be the criterion upon which to base a decision as to whether or not any individual is a diabetic. Only individuals whose permeability is near 130 are logical cases to follow by urinary examinations, for in such cases as soon as the blood sugar reaches 130 or above, sugar will appear in the urine.



TABLE XIV  
Relative Blood Volume as Calculated from the Corpuscle Volume.  
Non-Diabetic.

No.	Start			½ Hour			1 Hour			2 Hours			3 Hours			4 Hours			Rel. of Urine Output to Water Intake	
	Corp. Vol., %	Rel. Corp., %	Rel. Blood Vol., %	Corp. Vol., %	Rel. Corp., %	Rel. Blood Vol., %	Corp. Vol., %	Rel. Corp., %	Rel. Blood Vol., %	Corp. Vol., %	Rel. Corp., %	Rel. Blood Vol., %	Corp. Vol., %	Rel. Corp., %	Rel. Blood Vol., %	Corp. Vol., %	Rel. Corp., %	Rel. Blood Vol., %	Urine In-crease, %	Urine De-crease, %
1	46	100	100	43	93	107	43	93	107	44	95	105	44	95	105	33	71	129	.....	86
2	43	100	100	41	109	91	47	109	91	44	102	98	45	105	95	38	88	112	.....	24.2
4	38	100	100	42	110	90	39	102	98	42	110	90	40	105	95	39	102	98	4.3	.....
5	42	100	100	44	102	98	42	100	100	39	92	108	44	105	95	38	90	110	10.7	.....
6	35	100	100	38	109	91	34	97	103	37	106	94	31	88	112	.....	.....	.....	.....	96.5
7	39	100	100	42	108	92	35	90	110	35	90	110	32	82	118	39	100	100	24.6	.....
8	43	100	100	40	93	107	40	120	80	47	110	93	44	103	97	40	93	107	7.6	.....
9	41	100	100	40	98	102	41	100	100	47	115	85	47	114	86	41	100	100	.....	21.2
10	38	100	100	37	108	92	32	85	115	37	97	103	31	81	119	33	86	114	.....	27.5
11	46	100	100	44	95	105	41	90	110	45	97	103	45	98	102	.....	.....	1.2	.....	.....
12	37	100	100	36	97	103	30	81	119	35	95	105	32	86	114	34	92	108	.....	25.5
13	43	100	100	45	104	106	48	112	88	49	114	86	47	109	91	45	105	95	.....	11.8
14	40	100	100	46	115	85	48	120	80	44	110	90	40	100	100	.....	.....	.....	.....	68.5
15	45	100	100	46	102	98	43	96	104	44	98	102	37	82	118	41	91	109	30.5	.....
16	44	100	100	40	90	110	41	93	107	36	81	119	35	79	121	.....	.....	.....	.....	78.6
17	39	100	100	42	108	92	41	105	95	39	100	100	38	97	103	.....	.....	.....	.....	13.1
19	39	100	100	45	115	85	36	92	108	34	87	113	.....	.....	.....	35	89	111	.....	5.4
20	50	100	100	42	84	116	45	90	110	48	96	104	47	94	106	.....	.....	.....	.....	.....
21	43	100	100	39	90	110	41	95	105	46	107	93	44	102	98	41	96	104	15.7	.....
22	46	100	100	40	87	113	39	85	115	44	95	105	40	87	113	.....	.....	.....	.....	56.5
23	38	100	100	39	102	98	36	95	105	39	103	97	35	92	108	35	92	108	.....	39.2
24	43	100	100	43	100	100	37	86	114	36	84	116	40	93	107	.....	.....	.....	.....	43.4
25	44	100	100	47	107	93	43	98	102	47	107	93	44	100	100	55	125	75	.....	35.2
26	41	100	100	47	115	85	38	92	108	38	92	108	34	83	117	44	107	93	.....	50.7
27	46	100	100	37	80	120	36	78	122	39	85	115	39	85	115	40	87	113	.....	33.9

28	39	100	100	43	110	90	42	108	92	40	102	98	42	108	92	49	126	74	23.8	.....
29	30	100	100	40	105	95	35	92	108	38	100	100	57	150	50	50	131	69	.....	56.3
30	30	100	100	41	105	95	38	97	103	36	92	108	37	95	40	40	103	97	.....	5.8
31	39	100	100	36	92	108	41	105	95	41	105	95	39	100	100	41	105	95	.....	62.3
32	45	100	100	46	102	98	49	109	91	43	96	104	47	104	96	46	102	98	.....	40.7
33	46	100	100	43	93	107	45	98	102	41	91	109	43	93	107	47	102	98	.....	26.2
34	37	100	100	39	105	95	.....	.....	.....	37	100	100	40	108	92	.....	.....	.....	8.44	.....
35	43	100	100	37	86	114	40	93	107	40	93	107	42	97	103	.....	.....	.....	5.9	.....
36	40	100	100	29	73	127	31	78	122	30	75	125	26	65	135	30	75	125	.....	61.
37	.....	100	100	41	.....	.....	42	.....	.....	36	.....	.....	39	.....	.....	32	.....	.....	9.2	.....
38	42	100	100	40	95	105	45	107	93	40	95	105	44	105	95	40	95	105	.....	28.8
39	47	100	100	42	89	111	42	90	110	41	87	113	41	87	113	42	89	111	.....	2.3
40	49	100	100	38	78	122	47	96	104	42	86	114	46	94	106	41	84	116	.....	67.1
41	39	100	100	39	100	100	37	95	105	36	92	108	32	82	118	39	100	100	.....	50
42	38	100	100	41	108	92	41	108	92	33	87	113	41	108	92	39	103	97	.....	33.7
43	49	100	100	42	85	115	43	88	112	45	92	108	43	88	112	43	87	113	.....	73.5
44	51	100	100	45	88	112	52	102	98	53	104	96	49	96	104	40	78	112	.....	76.5
45	49	100	100	48	98	102	51	104	96	49	100	100	50	102	98	50	102	98	.....	41.2
46	41	100	100	42	102	98	39	95	105	40	98	102	40	98	102	36	88	112	.....	15.5
47	43	100	100	42	97	103	44	102	98	43	100	100	39	90	110	43	100	100	.....	7.8

The teaching still persists that the average renal permeability for sugar in normal individuals is at 170 mg. per 100 cc. The analysis of my data, however, as given in Table XIV, pp. 280-1, shows that in the great majority of normal cases the permeability level was below 170 mg. per 100 cc.

We must conclude therefore that the renal threshold is always an unknown factor until it has been identified by means of the glucose tolerance test or a series of simultaneous blood and urine determinations. Not until the renal threshold has been determined can we judge the significance of sugar in the urine of any individual.

*Wassermann reaction.* Wassermann tests with three separate antigens were made in all these cases, with positive reactions in 3—i. e., 3 per cent. as compared with 2.19 per cent. in the previous series. Syphilis has been a rare finding in a series of over 300 cases of diabetes which have been under my observation.

*Relative blood volume.* Only relative blood volume studies have been made, these being based on the corpuscle volume of the first blood taken, i. e., before the glucose was ingested. This volume is used as a basis of comparison for the subsequent examinations. The relative percentile increase or decrease of blood of the individual case, therefore, is based on this initial volume in that case. As an extreme example: if the corpuscle volume in a given case is estimated as 50 per cent. and at the end of one hour is 25 per cent., we conclude that during that period the plasma volume has increased from 50 to 75%, i. e., has increased to 150 per cent. of its original volume. It is on this basis that the appended tables have been calculated. (Tables XIV-XIX.)

The general comparison between the diabetic and the non-diabetic cases would indicate that in both the decrease in the corpuscle volume, or the increase in plasma which in turn signifies a retention of water in the blood stream, is the most marked feature. This is especially emphasized in the diabetic cases. I have reported in detail<sup>27</sup> work in which I have taken the red blood corpuscles of diabetic and of non-diabetic individuals and exposed each specimen for different periods of time to a certain concentration of sugar in normal saline to see whether or not there was any difference in their permeability—their readiness to



"take in" sugar. I found that the corpuscles of diabetic cases did not take in the sugar as readily as those of the normals. This, no doubt, accounts partly for the decreased corpuscle volume noted in diabetics. On the other hand since the corpuscles of the non-diabetic readily take in sugar, and also water, they consequently increase in size and there results the increased corpuscle volume shown in the table. One would expect then, that as soon as the individual begins to excrete large quantities of urine, he would thus dehydrate his blood stream and the relative corpuscle volume would be increased. This sounds logical, but let us see whether or not it is the case. Let us take, for instance, certain cases in which the output of urine was much greater than the intake of water and see what happened to the corpuscle volume.

Case 83 corpuscle volume unchanged

" 82	"	"	"
" 74	"	"	decreased
" 64	"	"	"
" 63	"	"	"
" 62	"	"	"
" 51	"	"	"
" 48	"	"	"

On the other hand in the following cases the urine volume was markedly diminished:

Case 96 corpuscle volume decreased

" 95	"	"	"
" 93	"	"	"
" 87	"	"	"
" 86	"	"	unchanged
" 85	"	"	"
" 81	"	"	decreased
" 79	"	"	unchanged
" 69	"	"	"
" 67	"	"	"

In this last group of cases the corpuscle volume is approximately what one's a priori reasoning would indicate. But in the first group the reverse is the case. Theoretically when such large quantities of urine leave the body, one would expect the corpuscle volume to rise but instead it is diminished. Two possible explanations may be offered: (1) the fact mentioned above, viz., that the corpuscles of the diabetic individual are more or less

TABLE XV  
Relative Blood Volume as Calculated from the Corpuscle Volume.  
Diabetic.

No.	Start			½ Hour			1 Hour			2 Hours			3 Hours			4 Hours			Rel. of Urine Output to Water Intake	
	Corp. Vol., %	Rel. Corp., %	Rel. Blood Vol., %	Corp. Vol., %	Rel. Corp., %	Rel. Blood Vol., %	Corp. Vol., %	Rel. Corp., %	Rel. Blood Vol., %	Corp. Vol., %	Rel. Corp., %	Rel. Blood Vol., %	Corp. Vol., %	Rel. Corp., %	Rel. Blood Vol., %	Corp. Vol., %	Rel. Corp., %	Rel. Blood Vol., %	Urine In-crease, %	Urine De-crease, %
48	42	100	100	38	90	110	34	81	119	35	83	117	37	88	112	39	93	107	.....	.....
49	48	100	100	42	87	113	45	93	107	52	109	91	40	83	117	46	96	104	16	.....
50	45	100	100	55	122	78	48	106	94	48	107	93	45	100	100	46	102	98	.....	65.7
51	38	100	100	36	95	105	38	100	100	38	100	100	40	105	95	42	110	90	20	.....
52	41	100	100	28	68	132	37	90	110	30	73	127	47	115	85	36	88	112	.....	60.6
53	38	100	100	36	95	105	35	92	108	45	118	82	29	76	124	36	95	105	.....	7.7
54	41	100	100	38	92	108	41	100	100	38	93	107	38	93	107	43	105	95	.....	30
55	39	100	100	45	115	85	49	126	74	40	103	97	41	105	95	38	97	103	.....	49
56	46	100	100	45	98	102	55	119	81	44	95	105	47	102	98	47	102	98	.....	84
57	47	100	100	49	102	98	40	85	115	43	91	109	41	87	113	46	98	102	.....	11.7
58	41	100	100	47	114	86	44	107	93	45	110	90	43	105	95	45	110	90	.....	81
59	43	100	100	42	97	103	45	104	96	43	100	100	43	100	100	45	104	96	.....	19.5
60	48	100	100	45	94	106	45	94	106	42	88	112	54	112	88	51	106	94	.....	28.2
61	43	100	100	35	81	119	42	98	102	43	100	100	40	93	107	33	77	123	.....	49.2
62	36	100	100	36	100	100	36	100	100	32	89	111	33	92	108	32	89	111	79	.....
63	44	100	100	42	95	105	42	95	105	43	98	102	40	91	109	43	98	102	31.4	.....
64	45	100	100	45	100	100	40	89	111	39	86	114	40	89	111	51	114	86	45.3	.....
65	45	100	100	44	98	102	44	98	102	41	91	109	42	93	107	38	85	115	.....	12.1
66	33	100	100	.....	.....	.....	37	112	88	.....	.....	.....	.....	.....	.....	30	90	110	2.9	.....
67	46	100	100	43	94	106	44	96	104	42	91	109	43	94	106	41	90	110	.....	59.1
68	43	100	100	40	93	107	42	98	102	37	86	114	46	90	110	39	90	110	.....	24.5
69	45	100	100	47	105	95	48	106	94	46	102	98	46	102	98	45	100	100	.....	40.7
71	42	100	100	41	97	103	45	107	93	43	102	98	44	105	95	49	117	83	.....	26.4
72	43	100	100	43	100	100	35	81	119	38	88	112	37	86	114	35	81	119	.....	8.3
74	42	100	100	35	83	117	36	86	114	34	81	119	32	76	124	37	88	112	47.8	.....

75	46	100	100	51	111	89	44	96	104	50	109	91	45	98	102	38	82	118	11.1
76	44	100	100	47	106	94	43	98	102	40	91	109	41	93	107	39	89	111	60
77	46	100	100	51	111	89	34	74	126	45	98	102	48	105	95	40	87	113	3.8
78	41	100	100	41	100	100	41	100	100	42	102	98	42	102	98	39	95	105	1.4
79	37	100	100	44	119	81	40	108	92	38	102	98	41	110	90	38	102	98	42.6
80	42	100	100	38	90	110	39	92	108	30	71	129	39	92	108	43	102	98	19.6
81	44	100	100	41	93	107	37	84	116	37	85	115	35	80	120	33	75	125	68
82	39	100	100	43	110	90	41	105	95	39	100	100	42	108	92	42	108	92	40
83	44	100	100	41	93	107	47	107	93	45	102	98	44	100	100	44	100	100	148
84	51	100	100	41	80	120	44	86	114	42	82	118	44	94	106	44	94	106	17.7
85	47	100	100	47	100	100	45	95	105	45	96	104	44	102	98	44	94	106	40
86	47	100	100	50	107	93	44	94	106	40	85	115	48	102	98	44	94	106	66.8
87	47	100	100	47	100	100	45	96	104	47	100	100	37	79	121	38	81	119	44.8
88	39	100	100	33	85	115	34	87	113	37	95	105	40	102	98	34	87	113	27.4
89	37	100	100	34	92	108	34	92	108	32	86	114	34	92	108	35	94	106	23.5
91	34	100	100	40	118	82	40	118	82	38	111	89	42	123	77	36	106	94	57.2
92	48	100	100	49	102	98	44	92	108	45	94	106	40	83	117	35	73	127	11.1
93	52	100	100	44	85	115	53	102	98	46	89	111	40	77	123	42	81	119	49.2
95	41	100	100	40	98	102	36	88	112	35	85	115	33	80	120	34	83	117	40
96	45	100	100	47	105	95	41	91	109	45	100	100	42	93	107	40	89	111	44.7
97	40	100	100	42	105	95	38	95	105	39	97	103	43	108	92	40	100	100	30.7
98	43	100	100	42	98	102	41	95	105	35	81	119	40	93	107	31	72	128	15.2
99	36	100	100	33	92	108	34	94	106	33	92	108	34	94	106	35	97	103	37.4
100	.....	.....	.....	.....	.....	.....	46	.....	.....	40	.....	.....	34	.....	.....	41	.....	.....	57



impermeable to sugar and thus do not increase in size, (2) the conclusion that there must be a withdrawal of water from the tissues to replace the loss by the urine output. These findings regarding the variations in blood volume emphasize the same conclusion drawn from our observations of the variations in the renal permeability to sugar, viz., that we cannot make general rules, but that each case is a law unto itself and reacts in its own peculiar way, and that therefore our judgment of each case must be based upon these individual peculiarities.

### SUMMARY

1. The urine output during the period of observation was greater than the water intake in 19.2 per cent. (25.5 per cent. in the first series) of the diabetics and 31.3 per cent. (42 per cent. in the first series) of the non-diabetics. The urine output was smaller than the water intake in 90.8 per cent. (74.5 per cent. in the first series) of the diabetics, and in 68.7 per cent. (50 per cent. in the first series) of the non-diabetics.

2. In the non-diabetics the maximum increase in the blood sugar concentration appeared very promptly after the ingestion of glucose, in 58.1 per cent. (50.9 per cent. in the first series) in one-half hour; in 33.2 per cent. (36.8 per cent. in the first series) in one hour; and in only 8.3 per cent. in 2 hours. In the diabetics the rise in the blood sugar concentration was slow, as was also the return to the normal level. In only 3.8 per cent. (4.6 per cent. in the first series) did the maximum rise appear one-half hour after the ingestion of glucose; in 15.4 per cent. (32.5 per cent. of the first series) it appeared at the end of one hour; in 54 per cent. (49 per cent. of the first series) at the end of two hours; in 23 per cent. (13.9 per cent. of the first series) it appeared at the end of three hours and in 3.8 per cent. at the end of the fourth hour.

3. The maximum increase in blood sugar concentration in all the cases was reached at the end of one-half hour in 30 per cent. (31 per cent. of the first series) of the cases. The absolute height of the blood sugar percentage after the ingestion of glucose appears to have little or no significance. The most important point is the length of time which it takes for the re-establishment of the normal level. After the ingestion of 100 gm. of glucose, if the curve comes back to the normal level within three hours, the

individual is considered non-diabetic; if more than three hours is required he is considered diabetic; the cases in which the return to the normal level hovers about the 3 hour period are considered as in the prediabetic stage.

4. While final evidence cannot be offered to prove that a delayed rise in the blood sugar content is not due to lack of absorption from the stomach or intestine, yet we can discard this point as of no practical value, for if it takes from four to nine hours

TABLE XVI  
Plasma (Relative Total Blood) Volume During Glucose Tolerance Test.  
Non-diabetic.

Increase of Plasma						Decrease of Plasma					
At	10%	20%	30%	40%	50%	At	10%	20%	30%	40%	50%
½ Hr.	14	7	2	-----	-----	½ Hr.	16	3	-----	-----	-----
1 Hr.	21	5	2	-----	-----	1 Hr.	10	3	-----	-----	-----
2 Hrs.	18	7	1	-----	-----	2 Hrs.	11	2	-----	-----	-----
3 Hrs.	12	11	1	1	-----	3 Hrs.	12	1	-----	-----	1
4 Hrs.	7	9	2	-----	-----	4 Hrs.	9	-----	2	1	-----

Diabetic.

Increase of Plasma						Decrease of Plasma					
At	10%	20%	30%	40%	50%	At	10%	20%	30%	40%	50%
½ Hr.	19	6	-----	1	-----	½ Hr.	8	9	1	-----	-----
1 Hr.	21	9	1	-----	-----	1 Hr.	9	3	1	-----	-----
2 Hrs.	13	14	2	-----	-----	2 Hrs.	10	2	-----	-----	-----
3 Hrs.	14	8	4	-----	-----	3 Hrs.	13	2	1	-----	-----
4 Hrs.	14	13	4	-----	-----	4 Hrs.	11	2	-----	-----	-----

for the curve to return to the normal level, the patient is a diabetic regardless of what amount of sugar may remain in the stomach or intestine.

5. Glycosuria and hyperglycemia are two independent factors, either of which may be present alone, or they may be co-existent. The significance of glycosuria cannot be determined without a simultaneous blood sugar estimation.

TABLE XVII  
A Study of the Relation of Corpuscle Volume to Blood Sugar Concentration.  
Diabetic.

No.	B. Sug. 0 Hr.	C. V. %	% Inc.	% Dec.	B. Sug. 1 Hr.	C. V. %	% Inc.	% Dec.	B. Sug. 2 Hrs.	C. V. %	% Inc.	% Dec.	B. Sug. 3 Hrs.	C. V. %	% Inc.	% Dec.	B. Sug. 4 Hrs.	C. V. %	% Inc.	% Dec.
48	122	42	...	10	238	34	...	19	204	35	...	17	125	37	...	12	88	39	...	7
49	98	48	...	13	168	45	...	7	162	52	...	...	142	41	...	17	107	46	...	4
50	146	55	22	...	254	48	6	...	171	48	7	...	161	45	...	...	116	46	2	...
51	105	38	...	5	158	36	...	...	199	38	...	...	145	40	...	...	67	42	10	...
52	108	41	...	32	191	37	...	10	180	30	...	27	139	47	...	15	117	36	...	12
53	122	38	...	5	248	35	...	2	243	45	18	...	199	29	...	24	95	36	...	5
54	130	41	...	8	200	41	...	...	243	38	...	7	214	38	...	7	100	43	...	...
55	122	39	...	...	250	49	26	...	243	40	3	...	164	41	...	...	78	38	...	3
56	156	46	...	...	240	55	19	...	250	44	...	5	165	47	2	...	129	46	2	...
57	150	41	...	...	204	40	...	15	187	43	...	9	171	41	...	13	124	45	...	2
58	150	41	...	...	300	44	7	...	256	45	10	...	193	43	...	...	124	45	10	...
59	103	43	...	3	252	45	4	...	226	43	...	...	172	43	...	...	162	45	4	...
60	120	48	...	6	272	45	...	...	319	42	...	12	178	54	12	...	130	51	6	...
61	147	43	...	19	292	42	...	2	316	32	...	...	280	40	...	7	174	33	...	23
62	117	36	...	...	250	36	...	...	330	43	...	11	297	33	...	8	176	32	...	11
63	154	44	...	5	292	42	...	...	316	32	...	2	241	40	...	9	133	43	...	2
64	140	45	...	...	272	40	...	11	416	39	...	14	389	40	...	11	272	51	...	14
65	132	45	...	2	340	44	...	2	428	41	...	9	467	42	...	7	375	38	...	15
66	204	37	...	...	323	...	12	...	394	...	...	...	460	...	...	...	384	30	...	10
67	201	46	...	6	380	44	...	4	497	42	...	9	460	43	...	6	353	41	...	10
68	184	43	...	7	434	42	...	2	454	37	...	14	285	39	...	10	261	39	...	10
69	212	45	...	...	362	48	6	...	475	46	2	...	400	46	...	...	234	45	...	...
70	178	...	...	3	440	...	...	...	506	43	...	...	454	44	5	...	323	...	...	...
71	124	42	...	...	227	45	7	...	467	37	...	12	467	37	...	14	405	35	...	19
72	167	43	...	...	336	35	...	...	423	38	...	...	475	...	...	...	394	...	...	...
73	84	...	...	...	272	...	...	...	423	34	...	19	475	...	...	...	37	...	...	...
74	126	42	...	17	243	36	...	14	428	34	...	...	426	32	...	24	352	37	...	12
75	151	46	...	...	333	44	...	4	389	50	...	...	375	45	...	2	306	38	...	18
76	99	44	...	...	232	43	...	2	300	40	...	9	297	41	...	7	303	39	...	11
77	252	46	...	...	248	34	...	26	508	45	...	...	374	48	5	...	282	40	...	13
78	200	41	...	...	410	41	...	...	516	42	2	...	405	41	10	...	416	39	...	5
79	128	37	...	10	362	40	8	...	525	38	...	...	405	41	...	...	308	38	2	...
80	117	42	...	7	261	37	...	16	485	37	...	29	537	39	...	8	485	43	2	...
81	154	44	...	...	416	41	...	...	366	35	...	15	366	35	...	20	326	23	...	25
82	206	39	...	...	460	47	...	...	475	39	...	...	428	42	...	...	362	42	...	...
83	280	44	...	7	460	47	...	...	475	45	...	...	380	44	...	...	313	44	...	...
84	234	51	...	20	460	44	...	14	500	42	...	18	401	...	...	...	324	...	...	...
85	277	47	...	...	428	45	...	5	428	45	...	4	323	44	...	6	280	44	...	6
86	206	47	...	...	454	44	...	6	543	40	...	15	357	37	2	...	306	44	...	...
87	191	47	...	...	344	45	...	...	423	47	...	...	344	37	...	21	277	38	...	19
88	236	39	...	15	333	33	...	13	375	37	...	5	330	40	2	...	313	34	...	13
89	282	37	...	8	423	34	...	...	563	32	...	14	575	34	...	8	460	35	6	...
91	306	33	...	...	482	40	18	...	544	38	...	...	442	42	23	...	374	36	...	...
92	270	48	...	...	326	44	...	8	475	45	...	6	428	40	...	17	333	33	...	27
93	222	53	...	15	428	53	2	...	400	46	...	11	374	40	...	23	334	42	...	19
95	91	41	...	2	206	36	...	...	294	35	...	15	300	33	...	20	313	34	...	17
96	130	45	...	...	174	41	...	...	265	42	...	...	244	42	...	7	288	40	...	11
97	319	40	...	...	416	38	...	...	552	39	...	3	490	43	8	...	440	40	...	...
98	221	43	...	2	280	41	...	5	365	35	...	19	434	38	...	7	584	35	...	28
99	272	36	...	8	444	34	...	6	852	42	...	...	544	33	...	...	600	41	...	3
100	357	...	...	...	750	46	...	...	...	...	...	...	652	34	...	...	...	...	...	...



6. There is no such thing as a fixed "normal renal threshold" (usually placed at 170 mg. per 100 cc.) for all individuals. Every individual is a law unto himself and may have either a high or a low renal threshold, an "individual threshold" which is normal for the individual in question. This threshold changes in cases of untreated diabetes and perhaps in nephritis cases also.

7. In this series of 100 cases, only 3 cases showed a positive Wassermann reaction (2.19 per cent in the former series).

### CONCLUSIONS

The findings in this second series of 100 cases in which glucose tolerance estimations have been made practically coincide with those of the first series. It would seem therefore that conclusions based on these findings are well founded, since there is no reason to believe that additional series would offer important variations.

Glucose tolerance estimations are of inestimable value in cases in which the diagnosis of diabetes is questionable, or in which a tangible proof for the diagnosis of diabetes is desired. The value of the glucose tolerance estimation in the diagnosis of diabetes may be compared with that of the X-rays in the diagnosis of fractures. The findings are clear-cut and their interpretation may be left to the physician. Especially in cases of spasmodic or persistent glycosuria, or in cases in which the fasting blood sugar is only slightly above the normal, the diagnosis of diabetes cannot be made with certainty without the aid of a glucose tolerance estimation. In obesity cases also,—in that transitory stage from the normal to the diabetic in which there is no fasting hyperglycemia so that the oncoming diabetes might readily be overlooked for months or even years, with increasing damage,—in such cases the glucose tolerance test will demonstrate unmistakably the lagging carbohydrate utilization. The most important issue in the whole problem is the timely discovery of the mild and unsuspected cases of diabetes—of the prediabetics. Once these cases are discovered, they can be kept in this prediabetic or mild diabetic status as useful members of society. By this means, therefore, preventive medicine may be employed to increasing advantage in the field of diabetes.

TABLE XVIII  
The Relation of Corpuscle Volume to Blood Sugar Concentration.  
Non-Diabetic.

No.	B. Sug. 0 Hr.	C. V. %	% Inc.	% Dec.	B. Sug. 1/2 Hr.	C. V. %	% Inc.	% Dec.	B. Sug. 1 Hr.	C. V. %	% Inc.	% Dec.	B. Sug. 2 Hrs.	C. V. %	% Inc.	% Dec.	B. Sug. 3 Hrs.	C. V. %	% Inc.	% Dec.	B. Sug. 4 Hrs.	C. V. %	% Inc.	% Dec.
1	73	46	...	...	109	43	...	6	105	43	...	7	70	44	...	5	61	...	...	...	60	34	...	29
2	98	43	...	...	100	41	...	5	100	47	...	...	85	44	...	2	61	...	...	...	74	38	...	12
3	97	38	...	...	113	42	10	...	119	39	2	...	93	42	...	10	63	...	...	...	84	39	2	...
4	71	42	...	...	108	44	...	...	75	42	...	...	63	39	...	8	65	...	...	...	55	38	...	10
5	98	36	...	...	128	38	9	...	114	34	...	...	97	37	...	6	85	...	...	...	...	...	...	...
6	77	39	...	...	121	42	8	...	123	35	...	...	96	35	...	...	87	...	...	...	45	39	...	...
7	104	43	...	...	130	40	...	...	97	52	20	...	80	47	...	...	80	...	...	...	74	40	...	7
8	125	41	...	...	145	40	...	2	100	41	...	...	91	47	...	...	60	...	...	...	102	41	...	...
9	76	38	...	...	133	37	...	...	106	32	...	...	106	37	...	...	91	...	...	...	68	34	...	14
10	116	46	...	...	164	44	...	...	117	41	...	...	85	45	...	...	77	...	...	...	...	...	...	...
11	85	37	...	...	119	36	...	...	161	32	...	...	104	35	...	...	110	...	...	...	68	34	...	8
12	99	43	...	...	135	45	...	...	139	48	...	...	129	49	...	...	80	...	...	...	71	45	...	...
13	109	40	...	...	143	46	...	...	100	48	...	...	90	44	...	...	85	...	...	...	58	41	...	...
14	98	45	...	...	139	46	...	...	87	43	...	...	128	36	...	...	62	...	...	...	79	41	...	...
15	95	44	...	...	133	40	...	...	158	41	...	...	116	39	...	...	93	...	...	...	...	...	...	9
16	94	39	...	...	146	42	...	...	129	41	...	...	105	34	...	...	85	...	...	...	...	...	...	...
17	94	39	...	...	163	45	...	...	105	36	...	...	105	34	...	...	89	...	...	...	83	37	...	...
18	109	50	...	...	184	42	...	...	166	45	...	...	139	48	...	...	90	...	...	...	...	...	...	...
19	83	43	...	...	150	39	...	...	130	41	...	...	131	46	...	...	65	...	...	...	...	...	...	...
20	93	46	...	...	158	40	...	...	152	39	...	...	94	44	...	...	70	...	...	...	...	...	...	...
21	115	38	...	...	161	39	...	...	164	36	...	...	117	39	...	...	88	...	...	...	...	...	...	...
22	127	42	...	...	170	42	...	...	102	37	...	...	77	36	...	...	81	...	...	...	...	...	...	...
23	158	43	...	...	183	46	...	...	150	43	...	...	96	47	...	...	120	...	...	...	...	...	...	...
24	92	41	...	...	147	47	...	...	146	38	...	...	70	38	...	...	79	...	...	...	...	...	...	...
25	75	37	...	...	158	37	...	...	120	26	...	...	136	39	...	...	39	...	...	...	...	...	...	...
26	100	39	...	...	176	43	...	...	158	42	...	...	89	40	...	...	90	...	...	...	...	...	...	...
27	111	38	...	...	183	40	...	...	135	35	...	...	130	36	...	...	98	...	...	...	...	...	...	...
28	97	45	...	...	243	36	...	...	147	41	...	...	107	36	...	...	82	...	...	...	...	...	...	...
29	91	46	...	...	146	46	...	...	75	49	...	...	142	43	...	...	110	...	...	...	...	...	...	...
30	79	37	...	...	153	43	...	...	159	45	...	...	126	41	...	...	61	...	...	...	...	...	...	...
31	130	40	...	...	184	39	...	...	139	45	...	...	76	37	...	...	69	...	...	...	...	...	...	...
32	94	43	...	...	165	29	...	...	174	37	...	...	130	40	...	...	91	...	...	...	...	...	...	...
33	75	42	...	...	127	29	...	...	153	31	...	...	146	30	...	...	132	...	...	...	...	...	...	...
34	94	41	...	...	161	41	...	...	194	42	...	...	145	36	...	...	75	...	...	...	...	...	...	...
35	108	49	...	...	166	42	...	...	129	45	...	...	94	40	...	...	35	...	...	...	...	...	...	...
36	88	49	...	...	152	38	...	...	189	47	...	...	173	40	...	...	83	...	...	...	...	...	...	...
37	95	38	...	...	137	39	...	...	171	37	...	...	192	36	...	...	104	...	...	...	...	...	...	...
38	108	41	...	...	99	41	...	...	191	41	...	...	206	33	...	...	94	...	...	...	...	...	...	...
39	146	49	...	...	219	42	...	...	286	43	...	...	176	45	...	...	83	...	...	...	...	...	...	...
40	94	51	...	...	207	45	...	...	230	50	...	...	202	53	...	...	70	...	...	...	...	...	...	...
41	105	49	...	...	139	48	...	...	280	39	...	...	179	49	...	...	94	...	...	...	...	...	...	...
42	133	41	...	...	226	42	...	...	280	40	...	...	202	40	...	...	116	...	...	...	...	...	...	...
43	88	43	...	...	125	42	...	...	186	44	...	...	197	43	...	...	124	...	...	...	...	...	...	...

The indications for the tolerance test are as follows:

1. The repeated presence of glycosuria even when a fasting blood sugar is normal. When the fasting blood sugar level is high that of itself alone establishes the diagnosis of diabetes.

2. When the fasting blood sugar lies between 130 and 160 mg. per 100 cc. Some cases in which the blood sugar is 160 mg. per 100 cc. are not diabetic and the diagnosis, therefore, can be established only by the tolerance test.

3. Obesity, whether or not the fasting blood sugar is normal. In many cases of obesity in which the fasting blood sugar is normal the tolerance test gives a diabetic curve while in other obese cases the obesity is due to familial tendencies and not to diabetes. In such cases while it is reasonable that the weight be reduced the individual should be relieved of the fear of diabetes.

TABLE XIX  
Summary of Tables XVII and XVIII

		½ Hr.	1 Hr.	2 Hrs.	3 Hrs.	4 Hrs.
Non-diabetic.....	Increase	147	113	95	128	113
	Decrease	218	242	230	282	229
Diabetic.....	Increase	152	127	77	96	74
	Decrease	237	269	342	330	386

4. A familial history of diabetes. It is important that individuals presenting a familial history of diabetes be subjected to the tolerance test in order that those with a diabetic tendency may be protected from the development of the disease and that those who do not show such a tendency may be protected from fear of its development.

5. A blood sugar content of more than 120 mg. per 100 cc. three or more hours after the last meal. This is an important indication since a hyperglycemia will not persist in a normal individual for more than three hours after a meal. This would make it appear that the logical time for a blood sugar examination in a new case would be three hours after the last meal for a



TABLE XX

A Survey of the 100 Cases Submitted to the Glucose Tolerance Test.

Number	Sex	Age	Occupation	History of Diabetes in Family	Measles	Rheumatism	Scarlet Fever	Mumps	Typhoid	Tuberculosis	Tonsillitis	Influenza	Diphtheria	Pneumonia	Pleurisy	Malaria	Appendectomy	Fasting Blood Sugar	Glycosuria	Wassermann	Gain in Weight	Loss in Weight	Phenolsulphonphenalein Renal Test	Blood Pressure	Blood Urea	Blood Uric Acid	Plasma Chlorides	Blood Creatinin	Chief Complaint	Diagnosis of Case
1	M	46	Professor	0	0	0	0	0	0	0	0	0	0	0	0	0	0	73	0	0	0	0	0	18	108/70	1.1	595	1.7	Glycosuria	Normal
2	M	36	Electrician	0	0	0	0	0	0	0	0	0	0	0	0	0	0	82	0	0	0	0	0	30	120/70	2.9	605	1.3	Weakness	Hypopituitary
3	M	28	Technician	0	0	0	0	0	0	0	0	0	0	0	0	0	0	75	0	0	0	0	0	34	128/86	1.1	585	1.1	None	Normal
4	M	36	Salesman	0	0	0	0	0	0	0	0	0	0	0	0	0	0	97	0	0	0	0	0	15	116/80	2.3	595	1.2	Glycosuria	Normal
5	F	45	Stenographer	0	0	0	0	0	0	0	0	0	0	0	0	0	0	84	0	0	0	0	0	15	116/80	1.8	555	1.1	Glycosuria	Normal
6	F	26	Housework	0	0	0	0	0	0	0	0	0	0	0	0	0	0	98	0	0	0	0	0	33	120/80	1.6	585	1.1	Glycosuria	Colitis
7	F	45	Housewife	0	0	0	0	0	0	0	0	0	0	0	0	0	0	77	0	0	0	0	0	12	160/100	2.3	585	1.1	Polyuria	Diab. Insipid
8	M	21	Cook	0	0	0	0	0	0	0	0	0	0	0	0	0	0	104	0	0	0	0	0	8	120/75	9	525	1.1	None	Normal
9	M	16	Student	0	0	0	0	0	0	0	0	0	0	0	0	0	0	125	0	0	0	0	0	24	120/80	3.2	545	1.1	Amenorrhea	Hypopituitary
10	F	23	Teacher	0	0	0	0	0	0	0	0	0	0	0	0	0	0	76	0	0	0	0	0	12	120/80	1.6	515	1.8	Convulsions	Epilepsy
11	M	23	Laborer	0	0	0	0	0	0	0	0	0	0	0	0	0	0	116	0	0	0	0	0	24	164/100	3.8	555	1.8	Sw. Joints	Syphilis
12	F	32	Housewife	0	0	0	0	0	0	0	0	0	0	0	0	0	0	85	0	0	0	0	0	15	110/65	1.5	535	1.8	Freq. Urination	Cystitis
13	M	19	Student	0	0	0	0	0	0	0	0	0	0	0	0	0	0	99	0	0	0	0	0	21	116/76	2.1	575	1.3	Pain Joints	Arthritis
14	M	27	Business	0	0	0	0	0	0	0	0	0	0	0	0	0	0	109	0	0	0	0	0	24	116/76	4.8	635	2.8	Malaise	Normal
15	M	36	Business	0	0	0	0	0	0	0	0	0	0	0	0	0	0	98	0	0	0	0	0	38	116/76	2.3	595	2.8	Fatigue	Anemia
16	M	25	Salesman	0	0	0	0	0	0	0	0	0	0	0	0	0	0	95	0	0	0	0	0	15	130/76	2.3	605	1.3	Glycosuria	Normal
17	F	27	Housewife	0	0	0	0	0	0	0	0	0	0	0	0	0	0	97	0	0	0	0	0	18	100/70	2.	585	1.3	Glycosuria	Normal
18	M	8	Boy	0	0	0	0	0	0	0	0	0	0	0	0	0	0	113	0	0	0	0	0	42	116/76	1.5	615	1.3	None	Normal
19	F	30	Housewife	0	0	0	0	0	0	0	0	0	0	0	0	0	0	120	0	0	0	0	0	18	116/76	2.5	565	1.8	Glycosuria	Normal
20	M	50	Contractor	0	0	0	0	0	0	0	0	0	0	0	0	0	0	109	0	0	0	0	0	12	120/70	2.7	585	1.3	None	Normal
21	M	22	Bellhop	0	0	0	0	0	0	0	0	0	0	0	0	0	0	101	0	0	0	0	0	21	120/80	3.	605	1.2	Gas	Normal
22	F	17	Schoolboy	0	0	0	0	0	0	0	0	0	0	0	0	0	0	93	0	0	0	0	0	30	120/80	3.	605	1.2	Loss weight	Chr. Constipation
23	F	50	Housewife	0	0	0	0	0	0	0	0	0	0	0	0	0	0	115	0	0	0	0	0	20	106/74	2.	625	1.3	Weak	Psychoneurosis
24	M	29	Lawyer	0	0	0	0	0	0	0	0	0	0	0	0	0	0	136	0	0	0	0	0	21	130/90	1.3	635	1.3	Glycosuria	Normal
25	M	28	Engineer	0	0	0	0	0	0	0	0	0	0	0	0	0	0	85	0	0	0	0	0	21	130/90	2.	605	1.5	None	Normal
26	M	32	Unholsterer	0	0	0	0	0	0	0	0	0	0	0	0	0	0	75	0	0	0	0	0	15	120/80	1.3	595	1.3	Glycosuria	Hyperacidty
27	M	24	Student	0	0	0	0	0	0	0	0	0	0	0	0	0	0	100	0	0	0	0	0	15	120/80	2.7	615	1.3	Enl. Joints	Arthritis
28	M	35	Merchant	0	0	0	0	0	0	0	0	0	0	0	0	0	0	111	0	0	0	0	0	15	140/80	3.	590	1.	Stomach Trouble	Achlorhydria
29	F	60	Housewife	0	0	0	0	0	0	0	0	0	0	0	0	0	0	107	0	0	0	0	0	30	130/90	3.	535	1.	Pruritus	Eczema
30	M	46	Carpenter	0	0	0	0	0	0	0	0	0	0	0	0	0	0	103	0	0	0	0	0	30	120/70	2.4	575	1.8	Diabetes	Normal
31	M	35	Druggist	0	0	0	0	0	0	0	0	0	0	0	0	0	0	97	0	0	0	0	0	18	120/70	1.8	555	1.8	Edema Legs	Varicose Vein
32	M	49	Housewife	0	0	0	0	0	0	0	0	0	0	0	0	0	0	91	0	0	0	0	0	12	125/75	1.8	585	1.7	Cough	Chr. Pharyngitis
33	F	39	Housewife	0	0	0	0	0	0	0	0	0	0	0	0	0	0	79	0	0	0	0	0	18	138/78	2.	605	1.9	Gout	Hypopituitary
34	F	38	Housewife	0	0	0	0	0	0	0	0	0	0	0	0	0	0	110	0	0	0	0	0	20	90/40	1.5	585	2.2	Stomach Trouble	Hypopituitary
35	M	38	Printer	0	0	0	0	0	0	0	0	0	0	0	0	0	0	130	0	0	0	0	0	20	140/70	3.	585	3.5	Dizziness	Chr. Endocardism
36	M	23	Schoolboy	0	0	0	0	0	0	0	0	0	0	0	0	0	0	94	0	0	0	0	0	17	130/80	2.7	565	1.2	Glycosuria	Normal
37	M	36	Cabinet	0	0	0	0	0	0	0	0	0	0	0	0	0	0	75	0	0	0	0	0	35/22	140/90	1.7	575	1.2	Increase B/P	Chr. Nephritis
38	M	37	Physician	0	0	0	0	0	0	0	0	0	0	0	0	0	0	88	0	0	0	0	0	9	120/70	2.2	575	1.9	Diabetes	Chr. Pharyngitis
39	M	38	Physician	0	0	0	0	0	0	0	0	0	0	0	0	0	0	95	0	0	0	0	0	30	140/70	2.5	545	1.0	Cancer	Ca. Rectum
40	M	32	Furniture	0	0	0	0	0	0	0	0	0	0	0	0	0	0	108	0	0	0	0	0	18	130/82	2.4	575	1.6	Weakness	Diab. Mellitus
41	M	66	Brewer	0	0	0	0	0	0	0	0	0	0	0	0	0	0	95	0	0	0	0	0	49	130/70	3.	545	1.3	Dizziness	Syphilis
42	M	18	Plumber	0	0	0	0	0	0	0	0	0	0	0	0	0	0	146	0	0	0	0	0	15	130/70	4.3	555	1.3	None	Pre Diab.
43	M	39	Business	0	0	0	0	0	0	0	0	0	0	0	0	0	0	94	0	0	0	0	0	15	130/70	2.7	575	1.1	Glycosuria	Normal
44	M	37	Tailor	0	0	0	0	0	0	0	0	0	0	0	0	0	0	105	0	0	0	0	0	9	210/100	3.	585	1.1	Paralysis	Hypertension
45	M	45	Physician	0	0	0	0	0	0	0	0	0	0	0	0	0	0	133	0	0	0	0	0	30	140/74	2.5	600	1.8	Loss of Weight	Pre Diabetic
46	M	55	Merchant	0	0	0	0	0	0	0	0	0	0	0	0	0	0	88	0	0	0	0	0	27	140/74	2.5	600	1.8	Listlessness	Pre Diabetic
47	M	55	Movie Man	0	0	0	0	0	0	0	0	0	0	0	0	0	0	122	0	0	0	0	0	21	140/74	2.5	600	1.8	Listlessness	Pre Diabetic
48	M	28	Lawyer	0	0	0	0	0	0	0	0	0	0	0	0	0	0	122	0	0	0	0	0	21	140/74	2.5	600	1.8	Listlessness	Pre Diabetic

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fasting blood sugar might be normal because of the protraction of time, 12 to 14 hours, since the last meal on the preceding evening.

It may be noted that sufficient practical information may be secured by a test of the fasting blood sugar and a second test made three hours after the patient has received 100 gms. of glucose by mouth. The blood sugar level in the second test will determine whether or not the patient is a diabetic—if it is high he is a diabetic, if it is normal or below normal, he is a non-diabetic.

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CHART I.  
PERCENTAGE OF URINARY OUTPUT TO WATER INTAKE  
NONDIABETIC

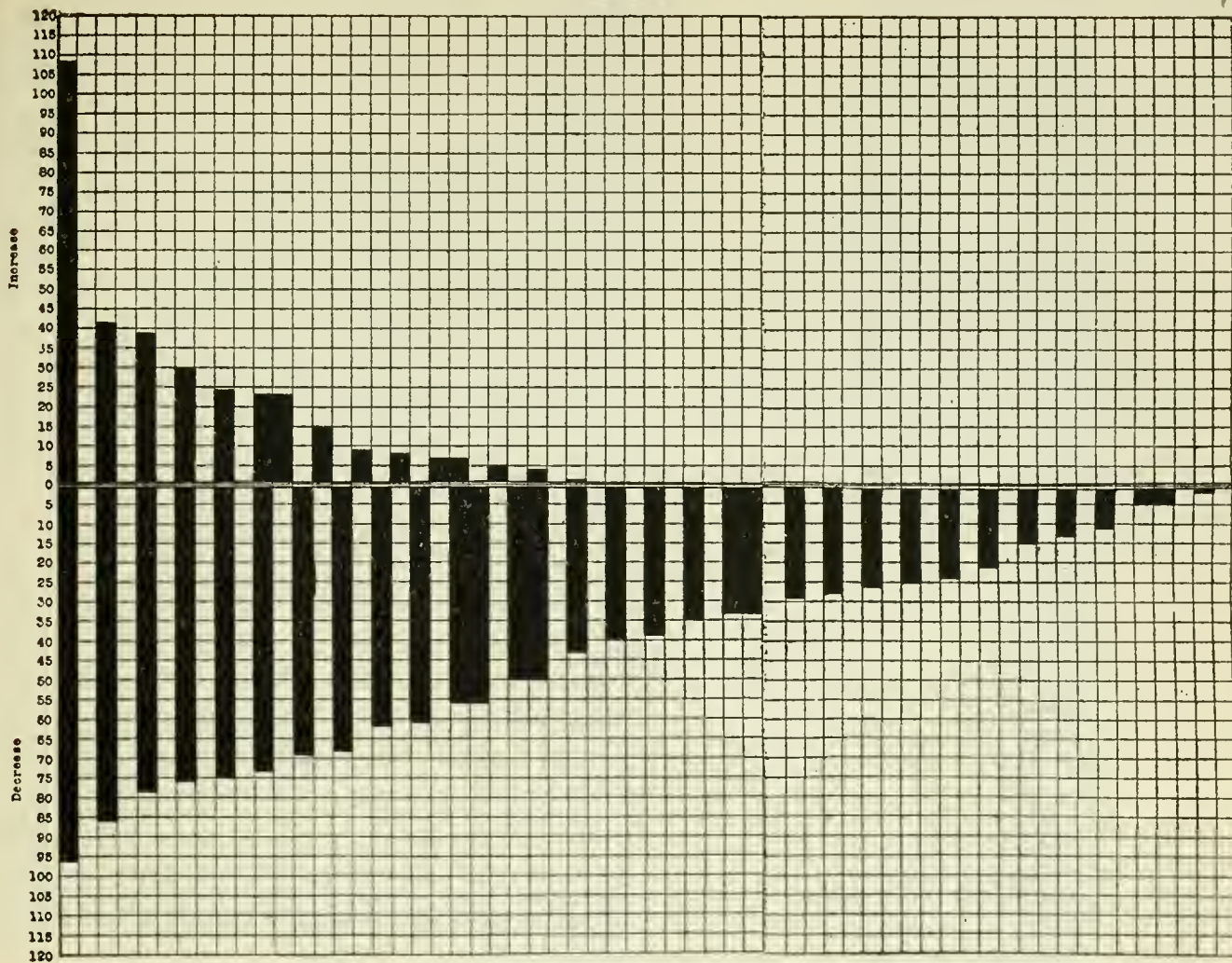
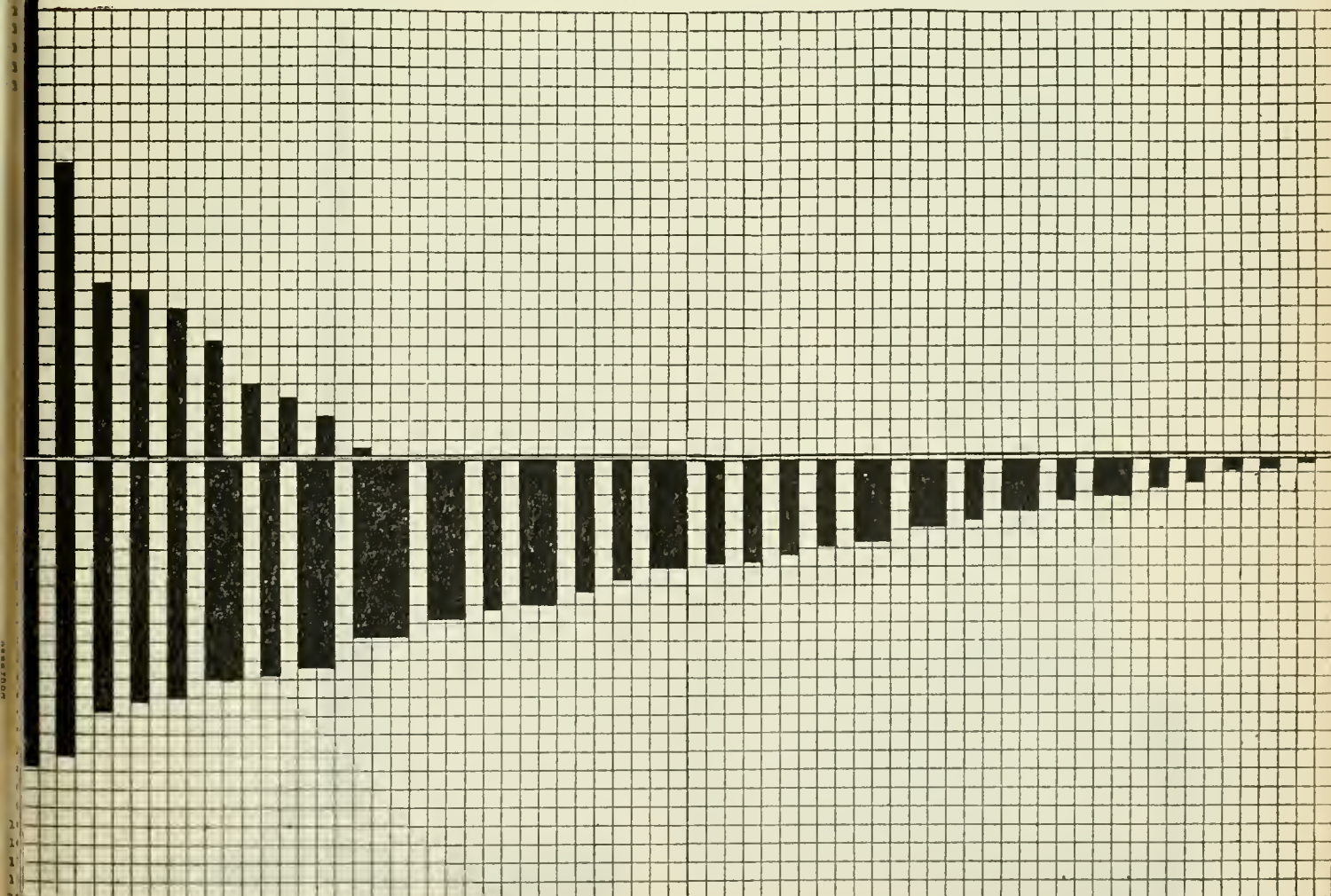


CHART II.  
PERCENTAGE OF URINARY OUTPUT TO WATER INTAKE  
DIABETIC





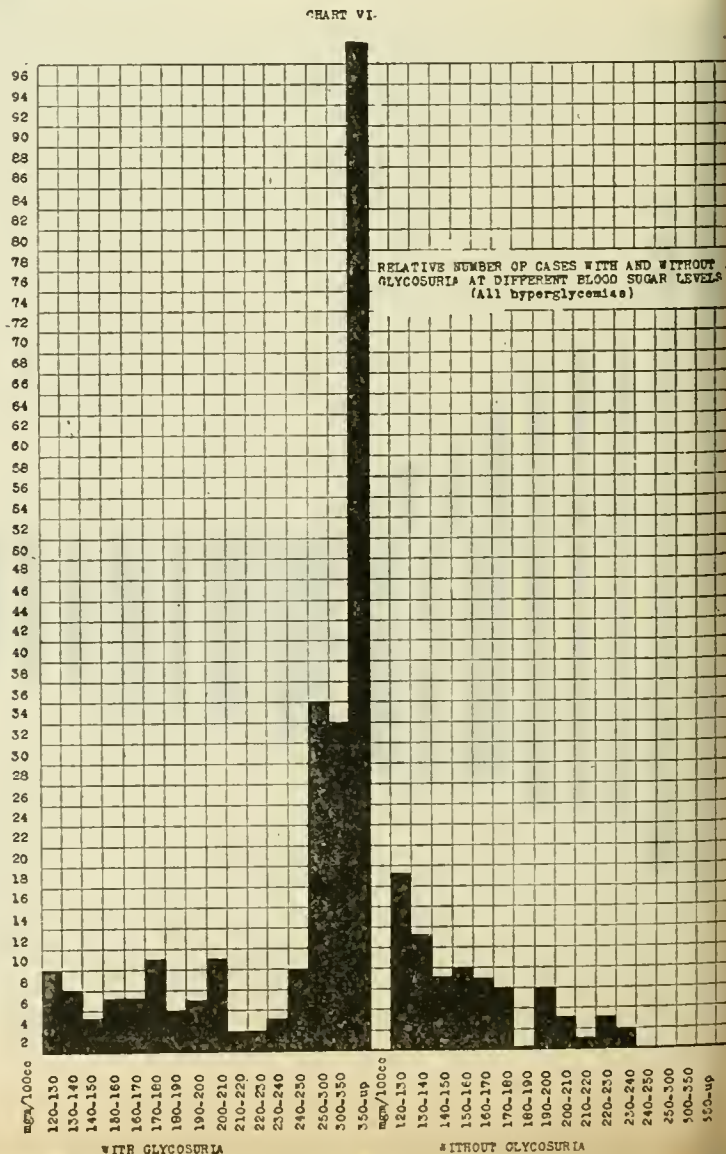
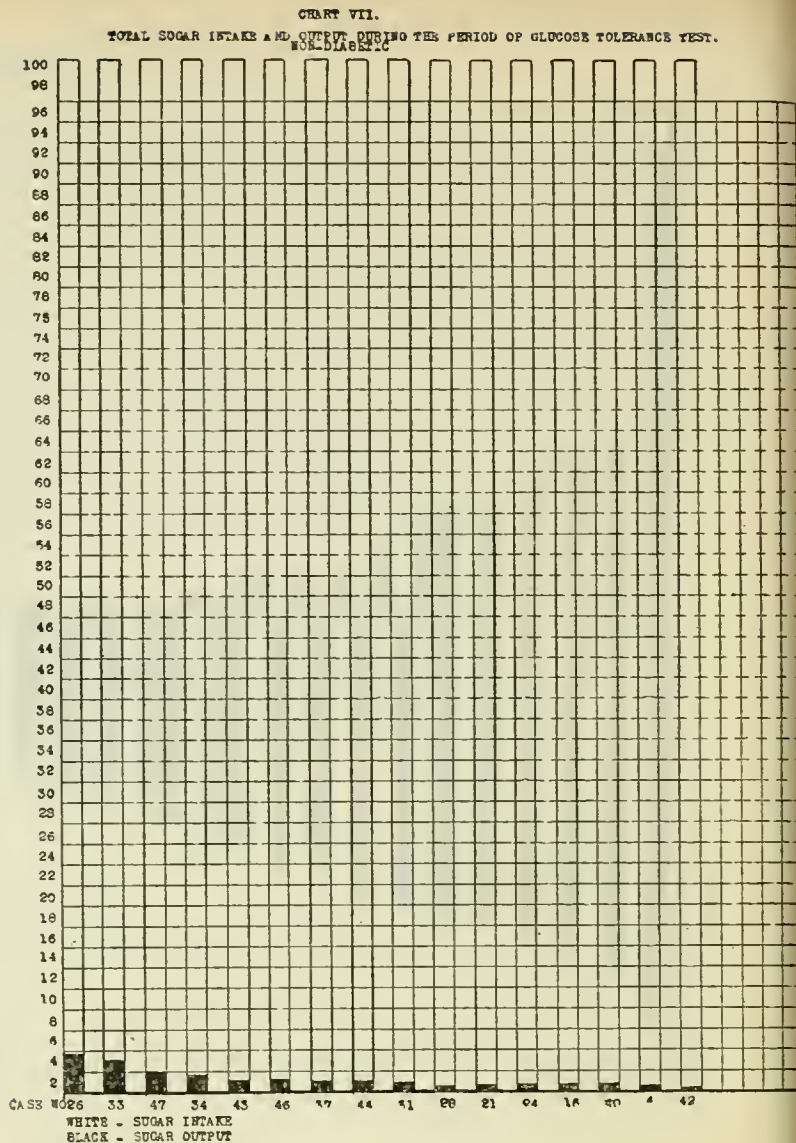
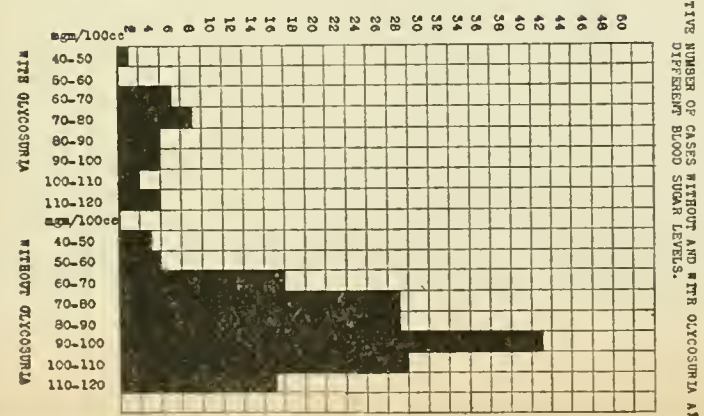
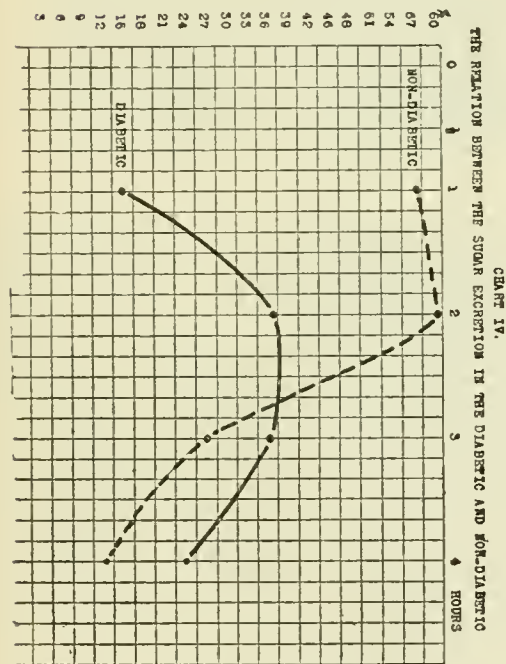
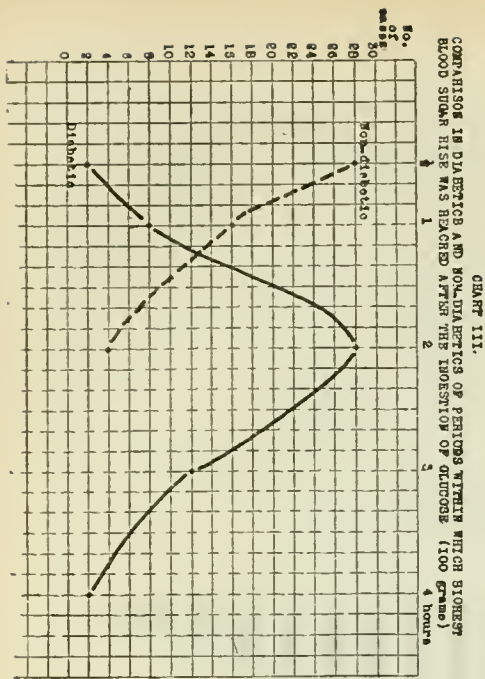
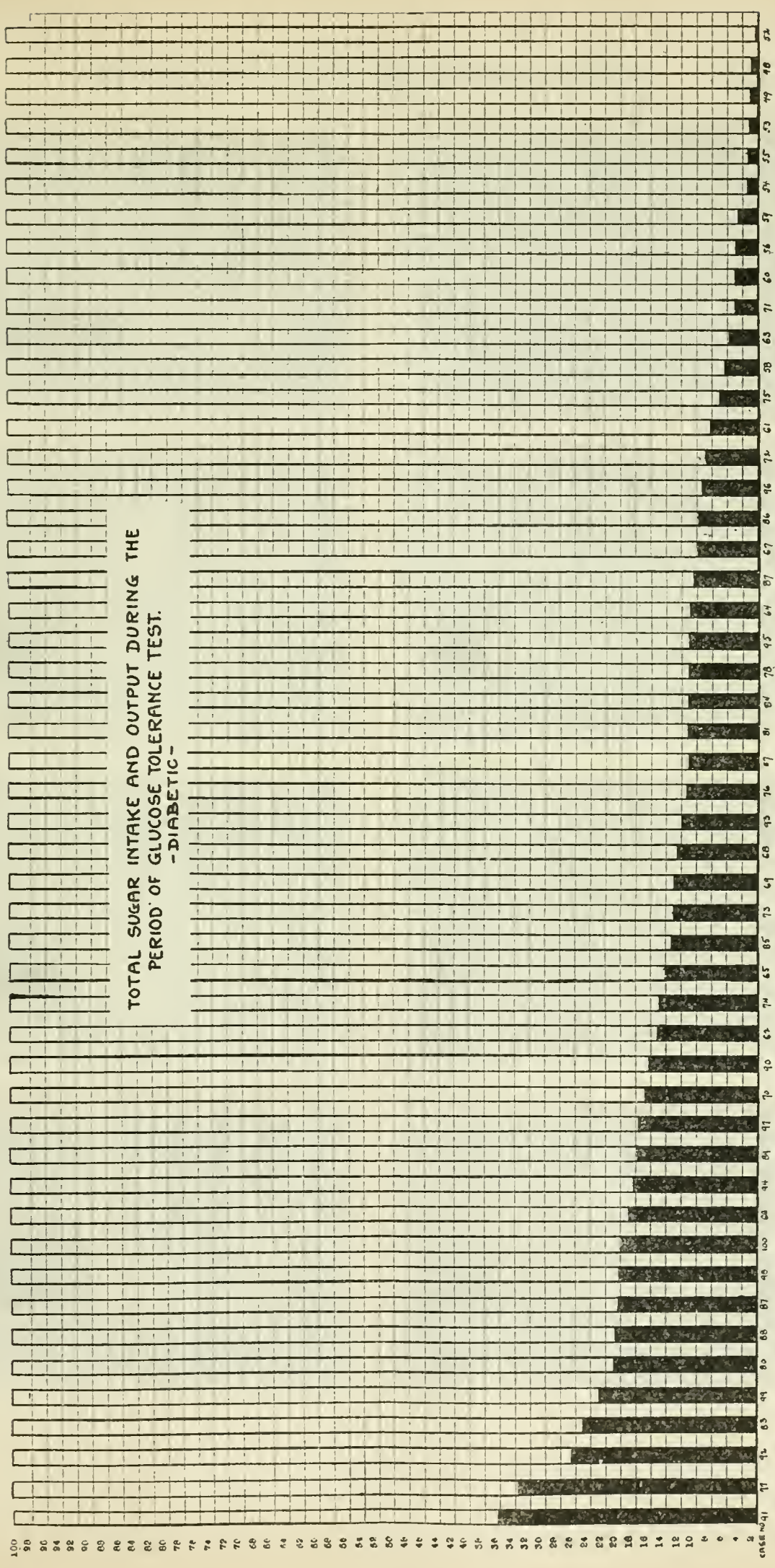
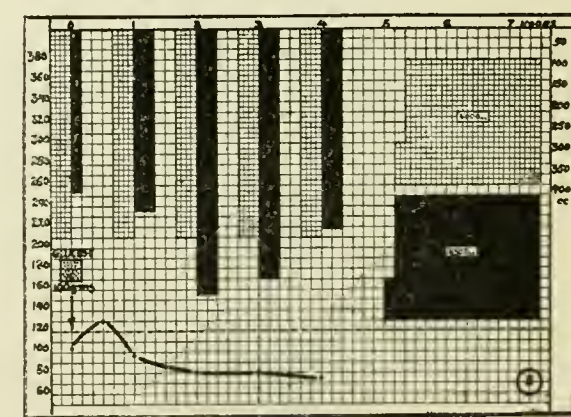
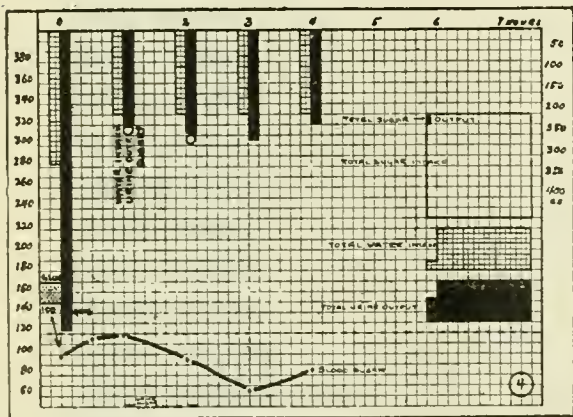
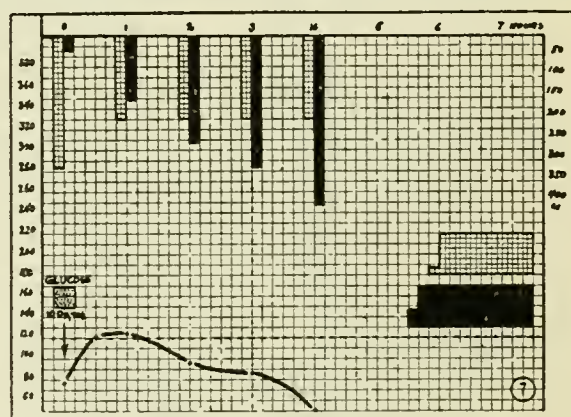
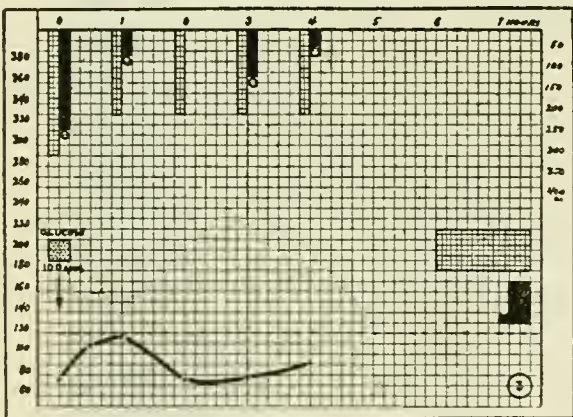
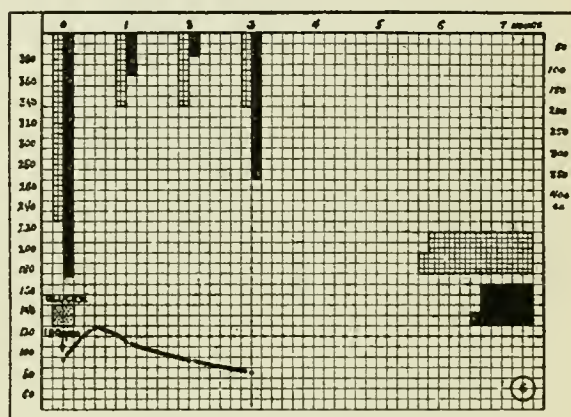
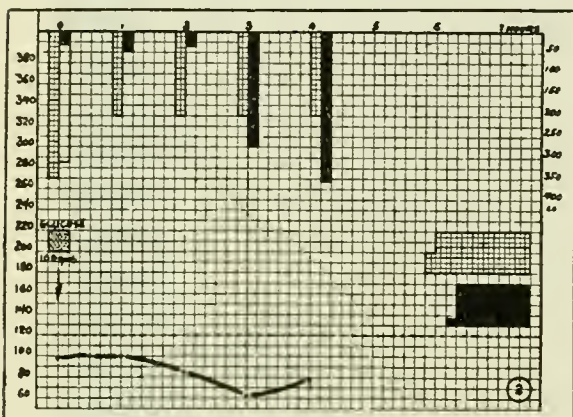
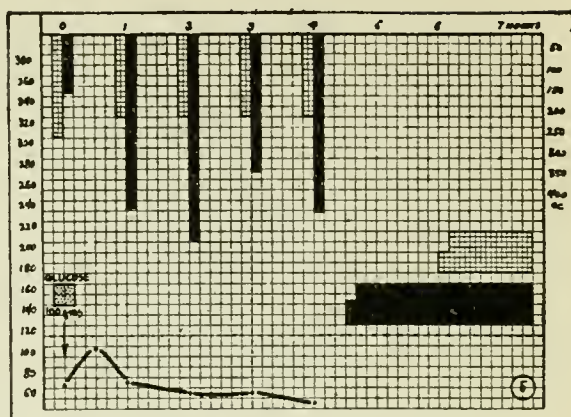
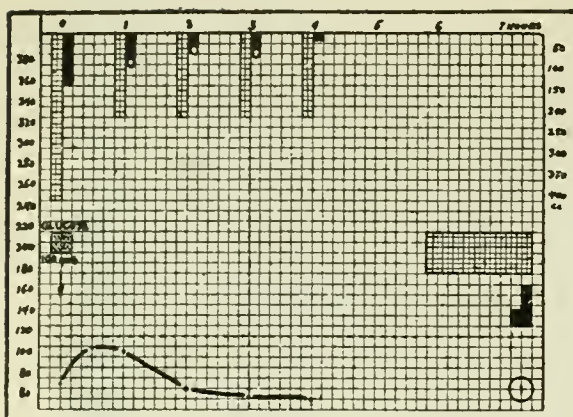




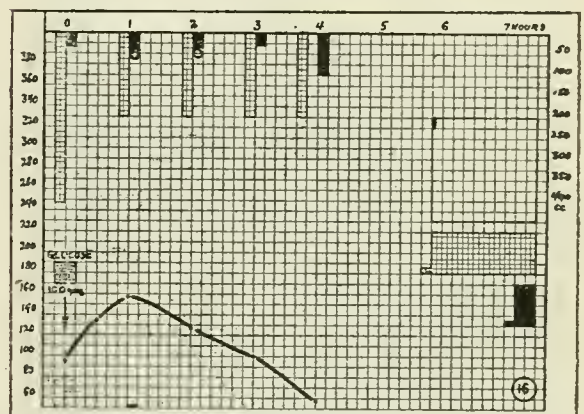
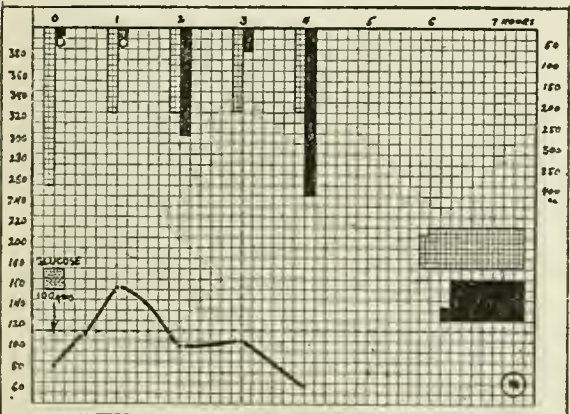
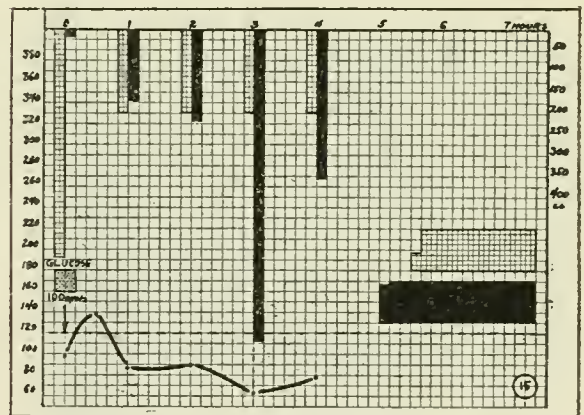
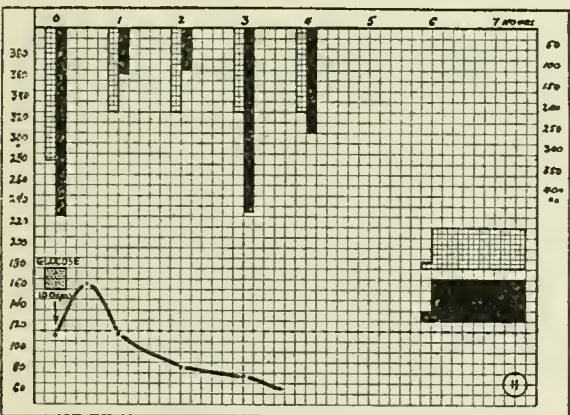
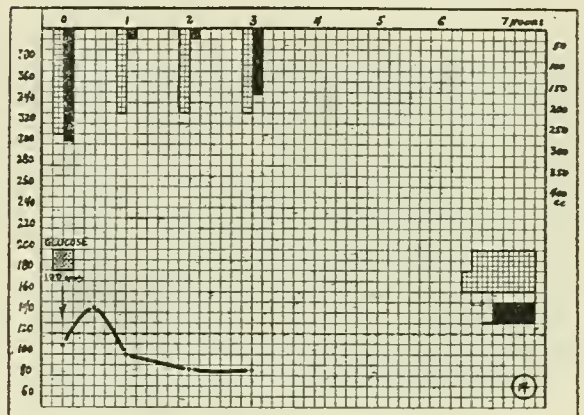
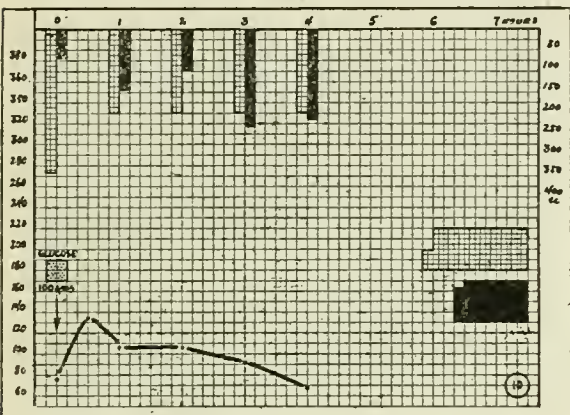
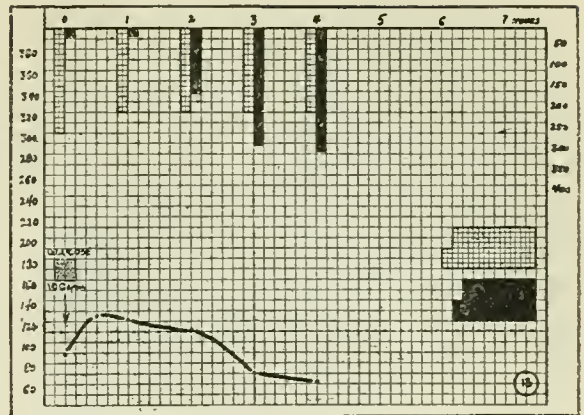
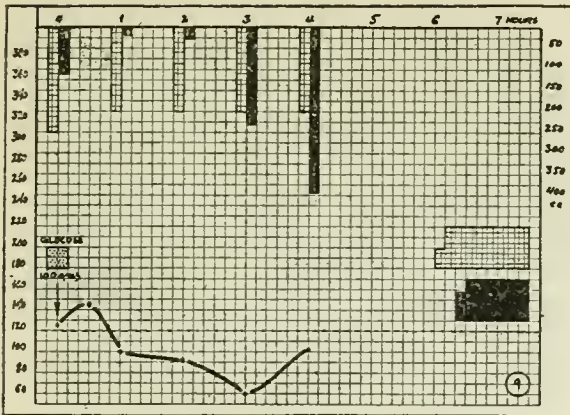
CHART VIII



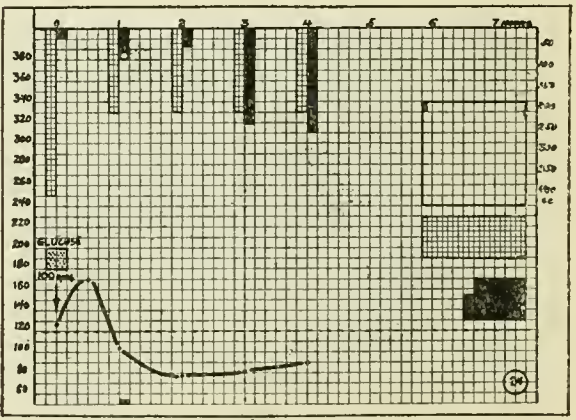
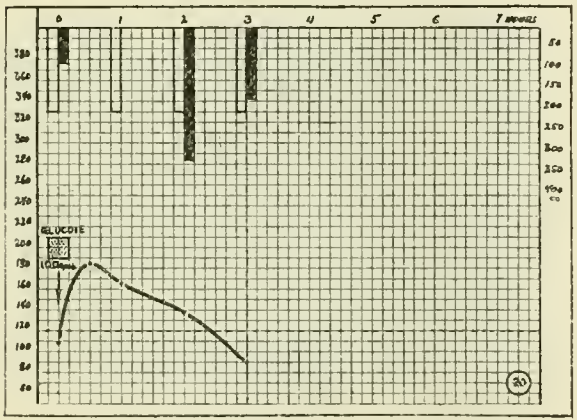
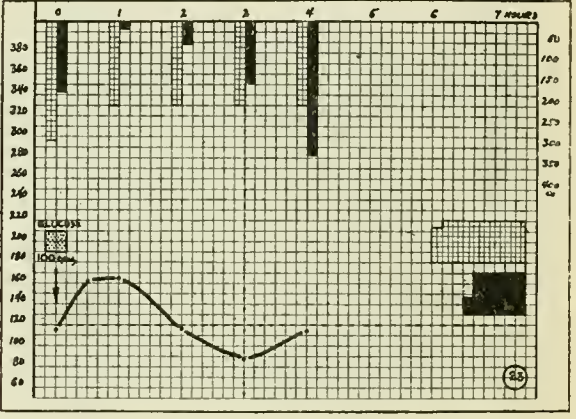
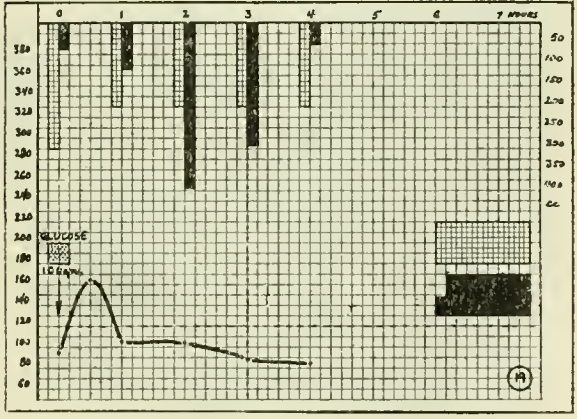
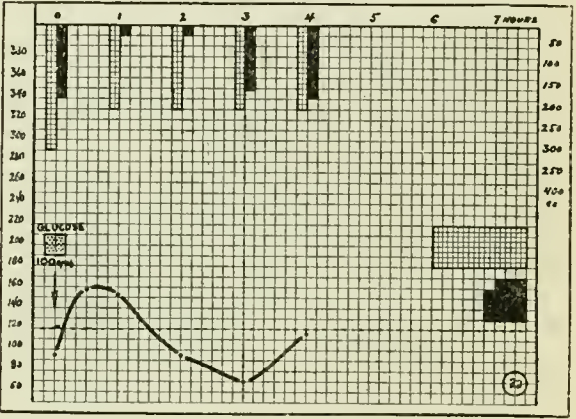
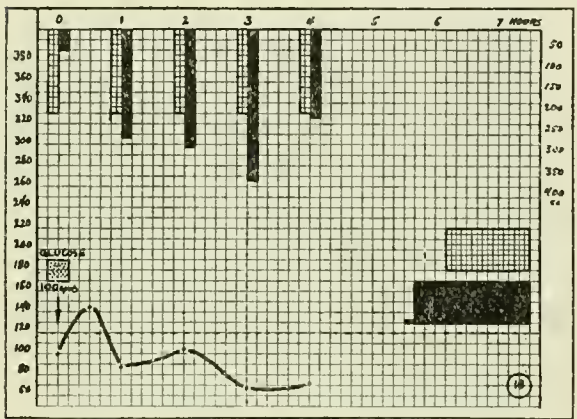
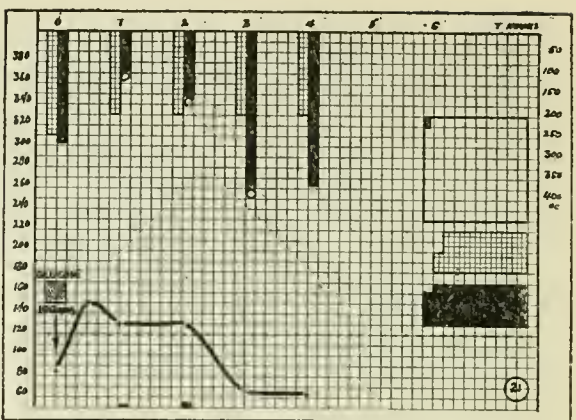
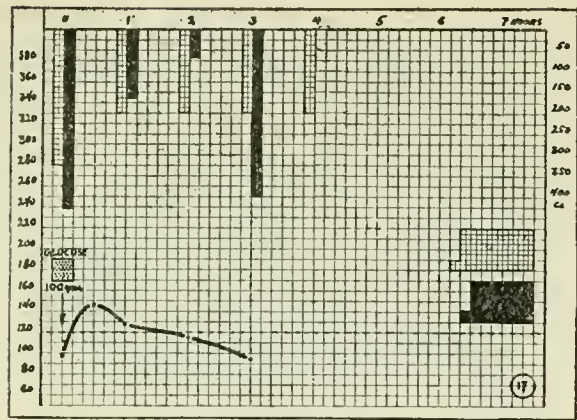




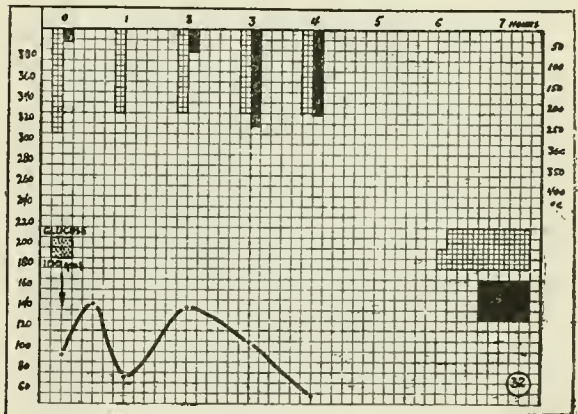
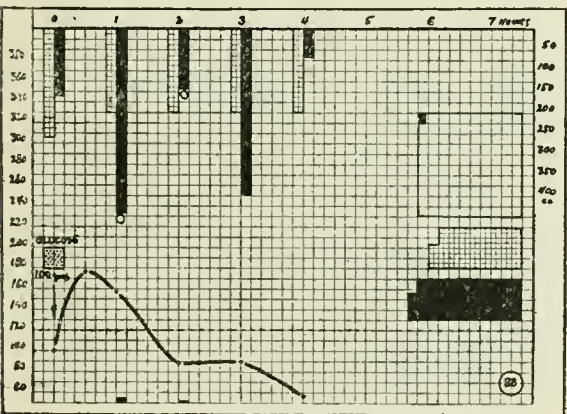
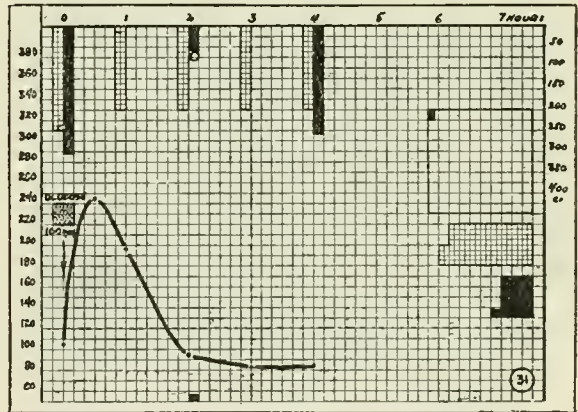
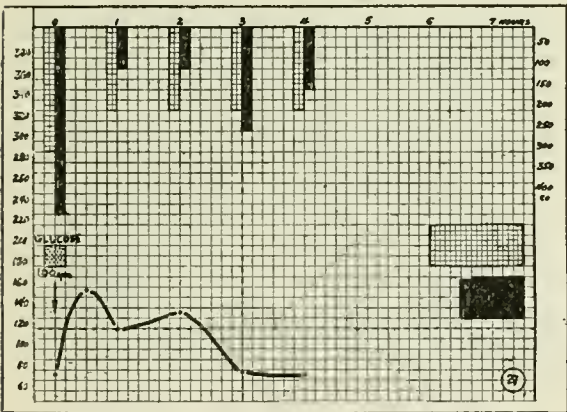
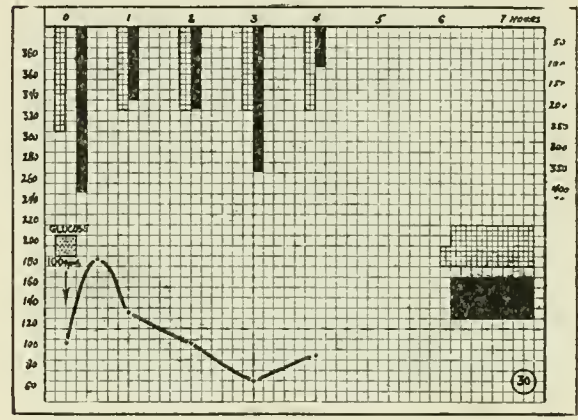
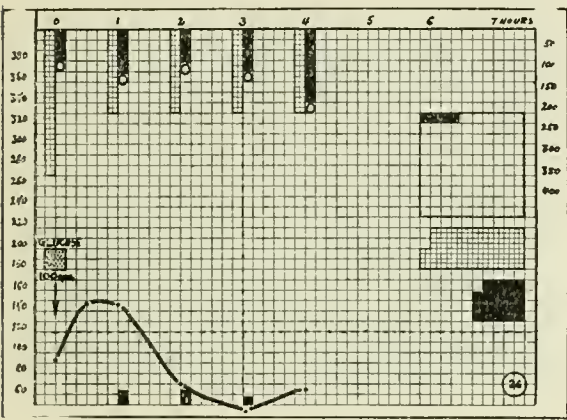
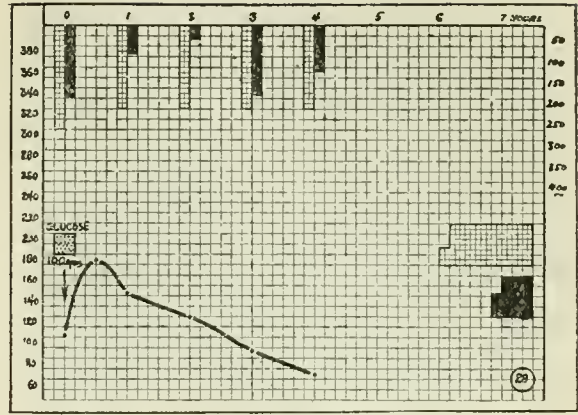
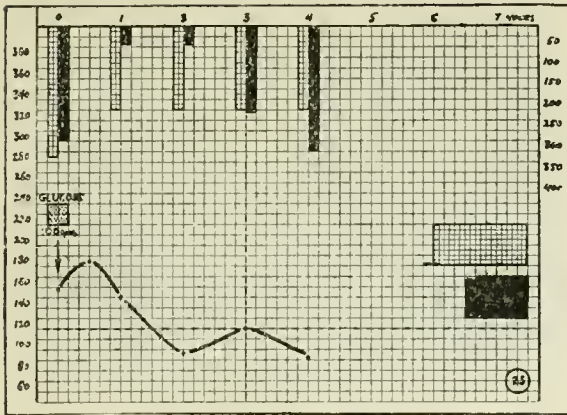




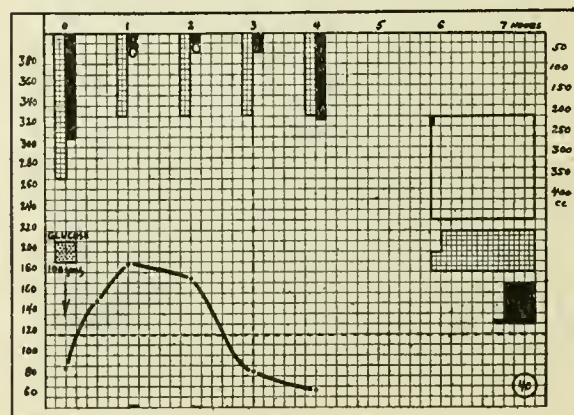
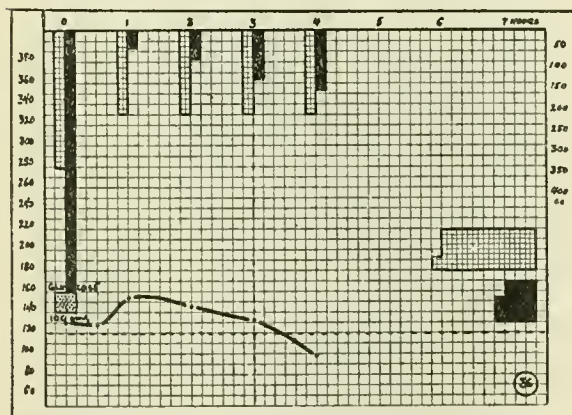
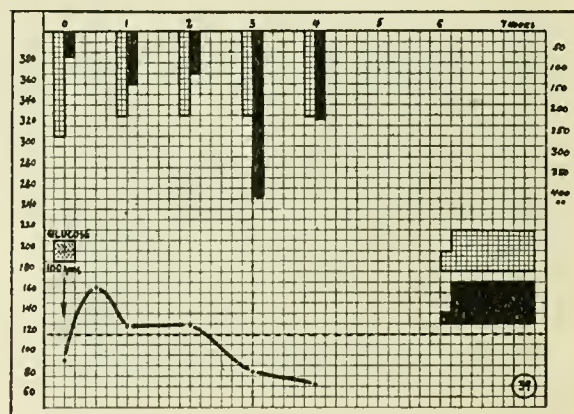
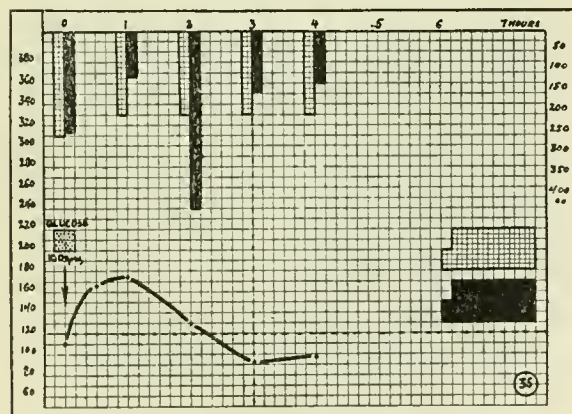
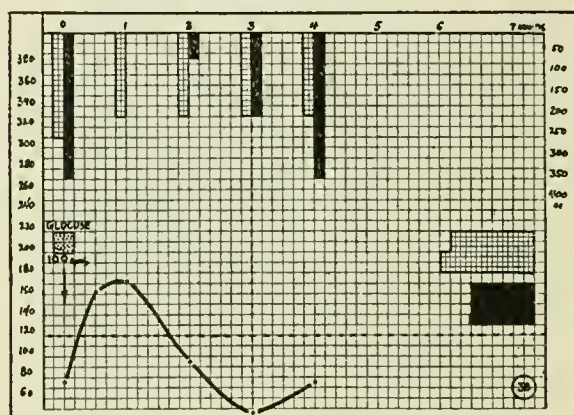
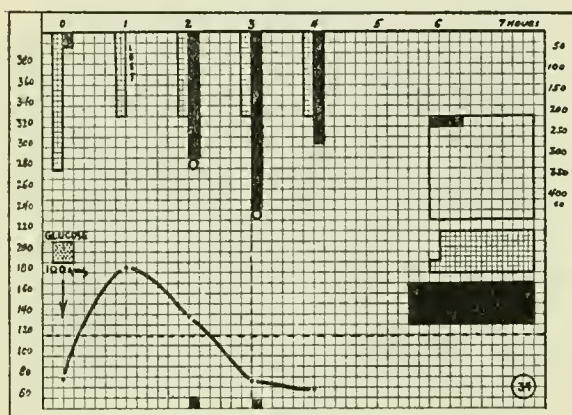
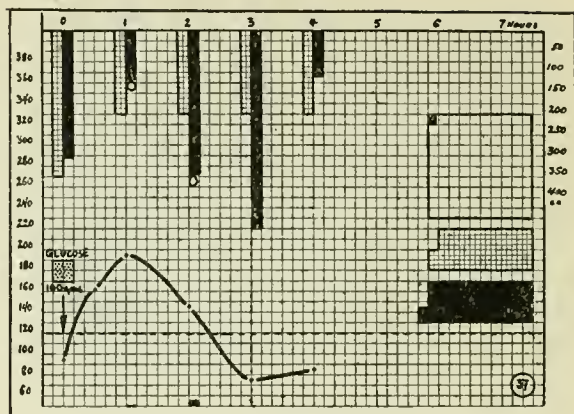
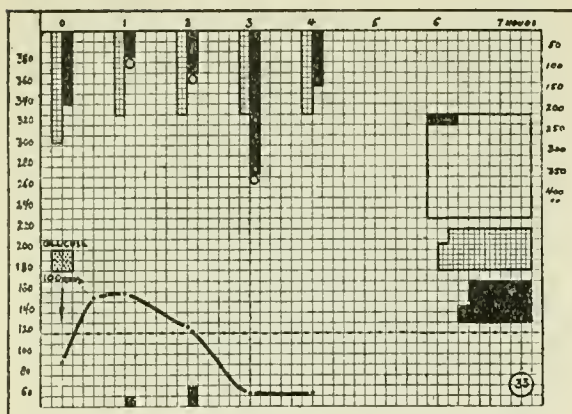




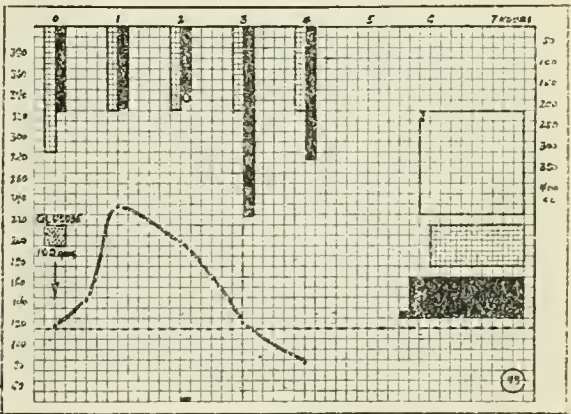
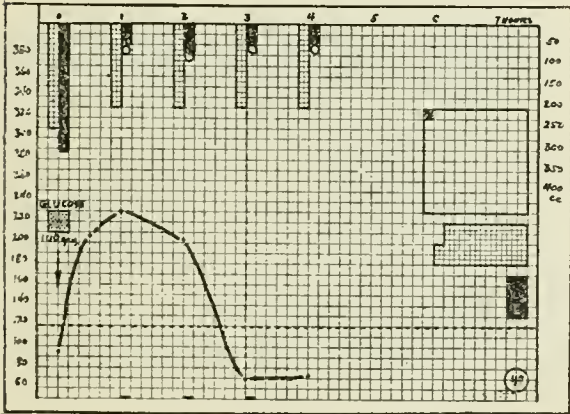
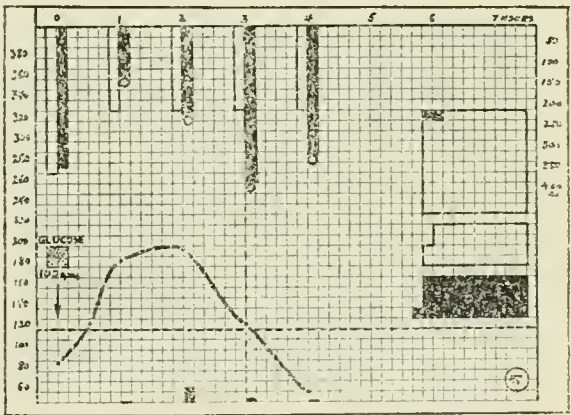
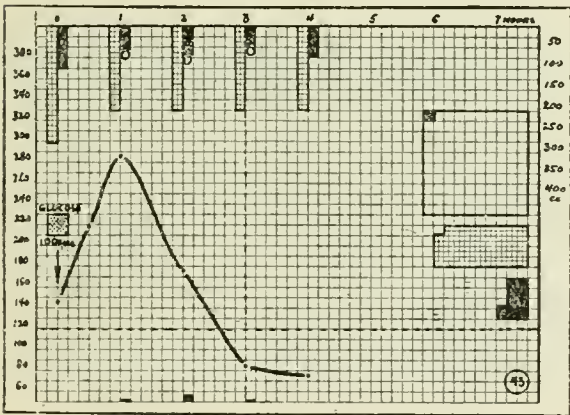
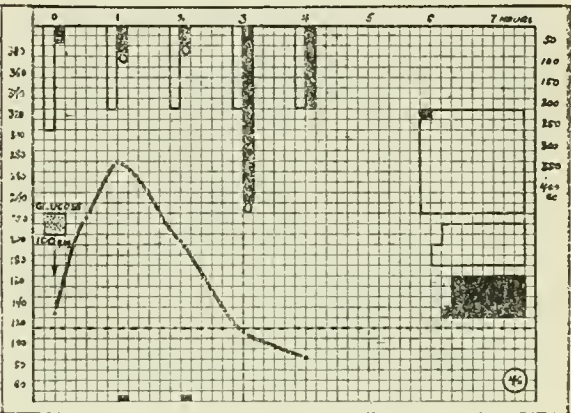
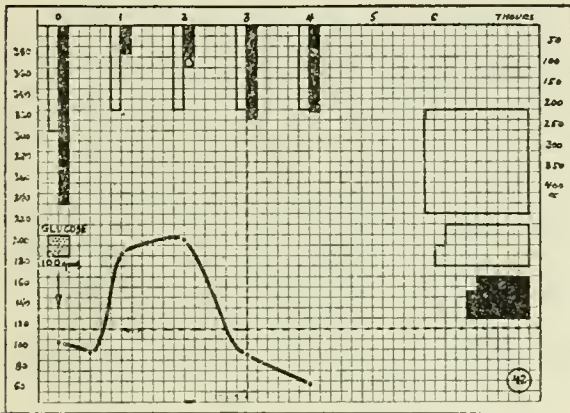
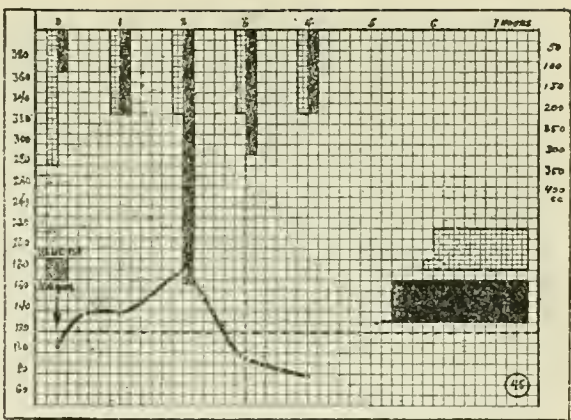
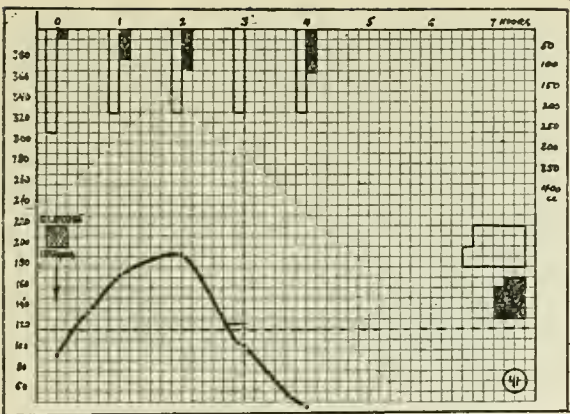




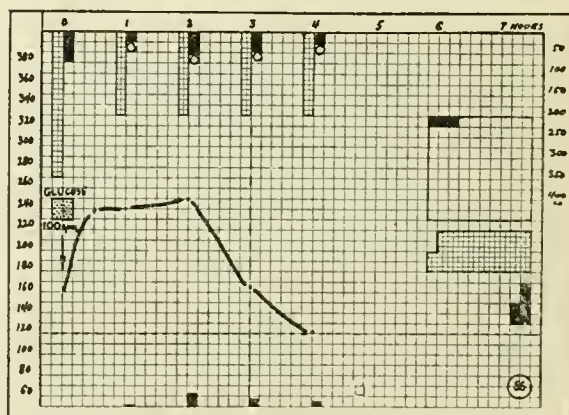
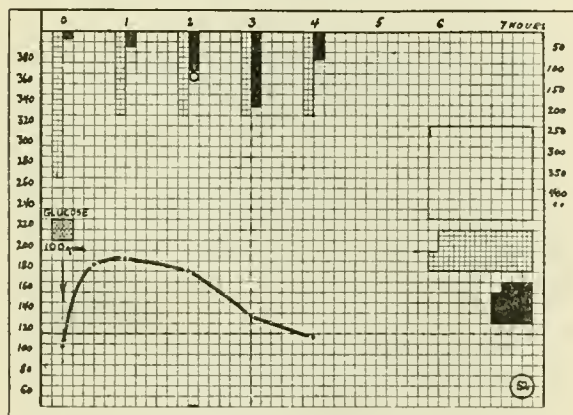
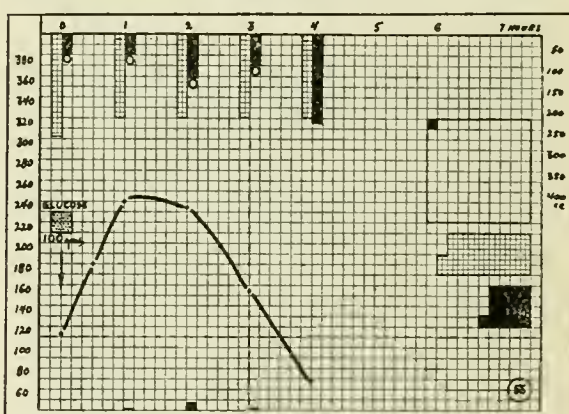
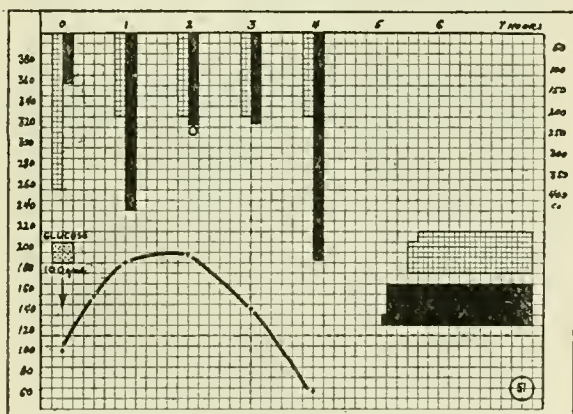
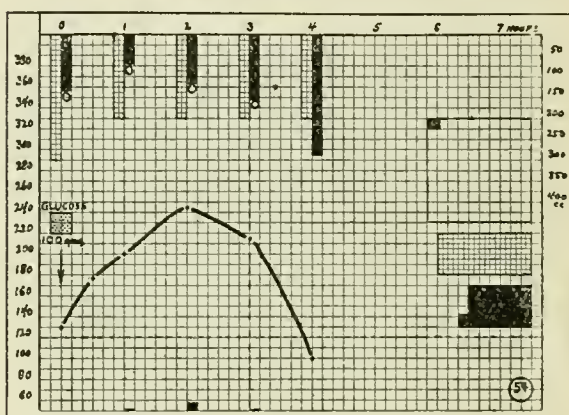
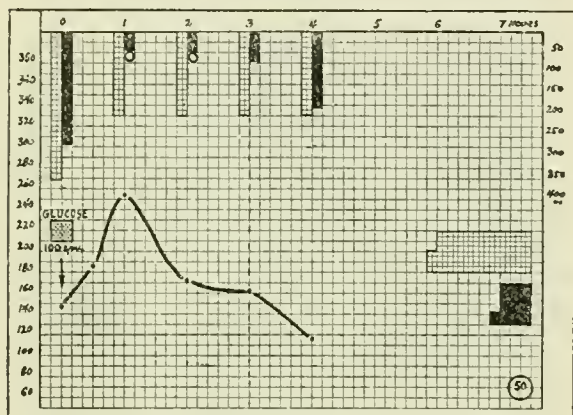
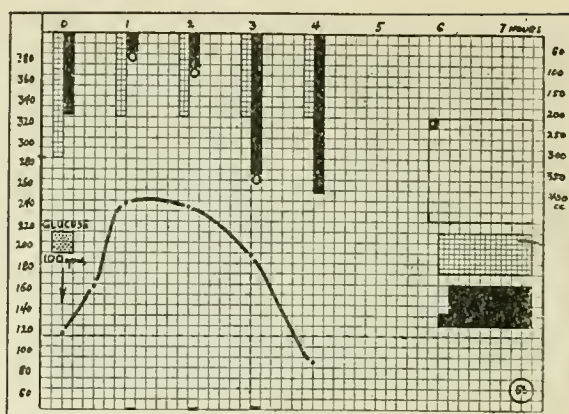
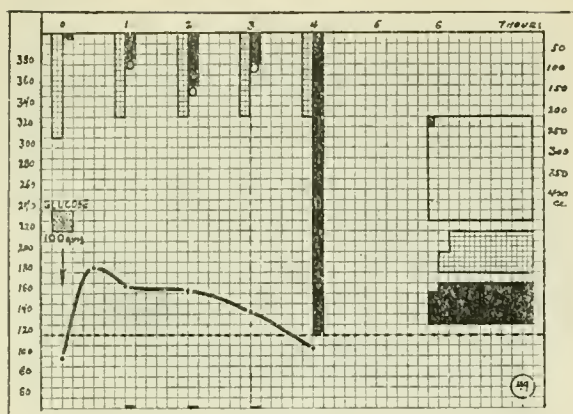




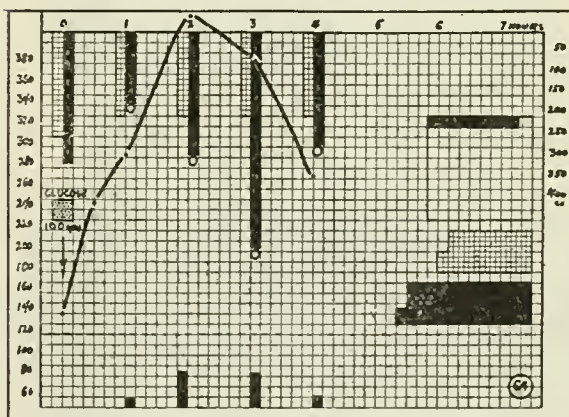
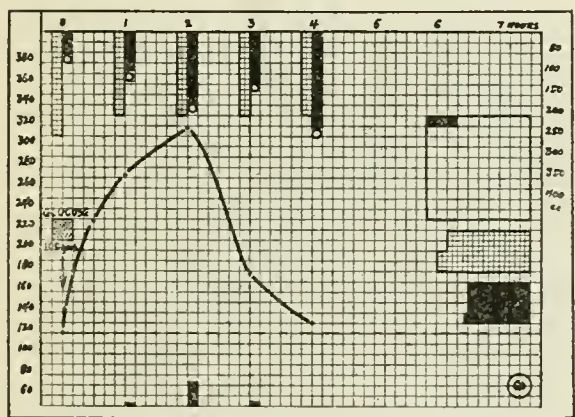
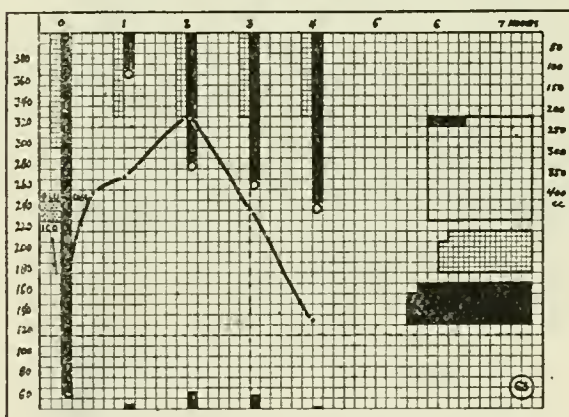
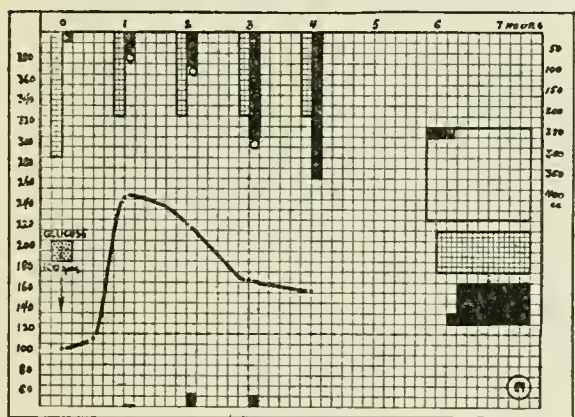
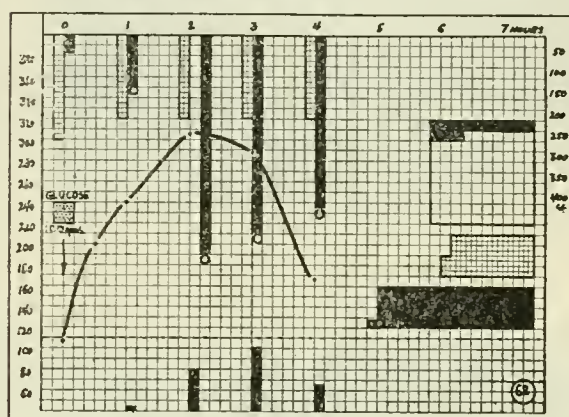
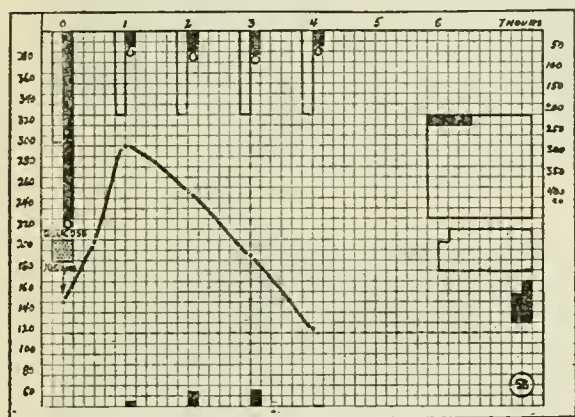
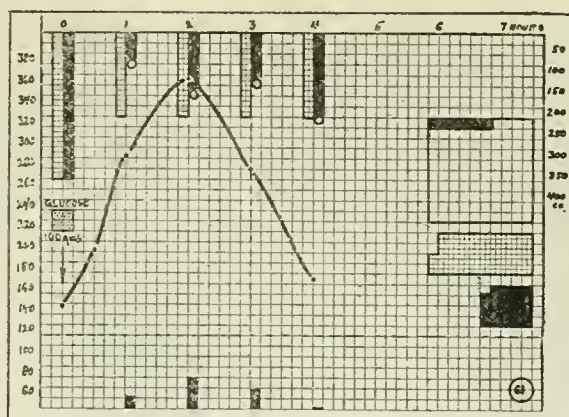
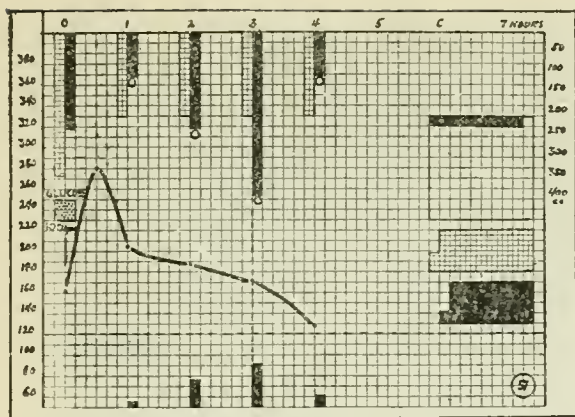




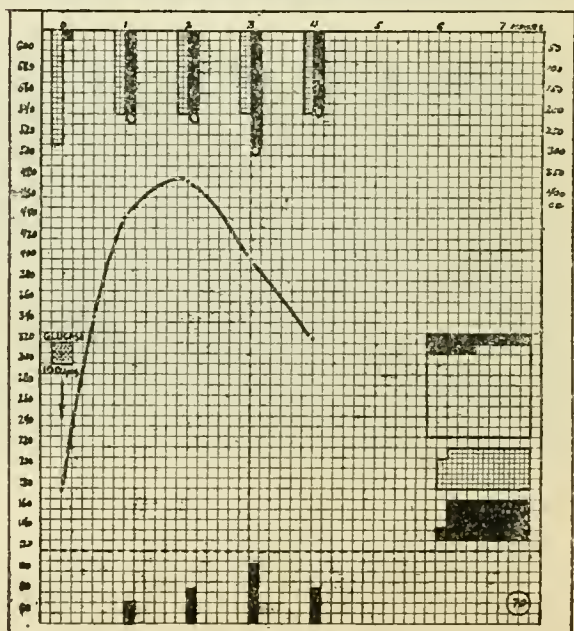
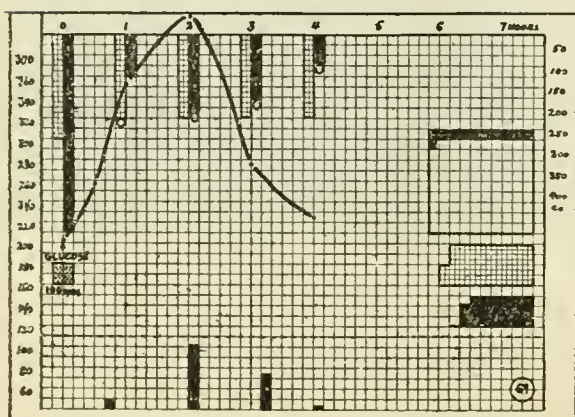
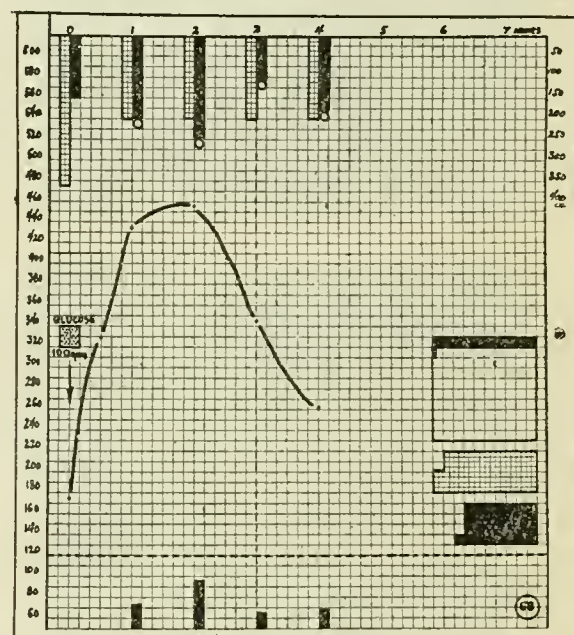
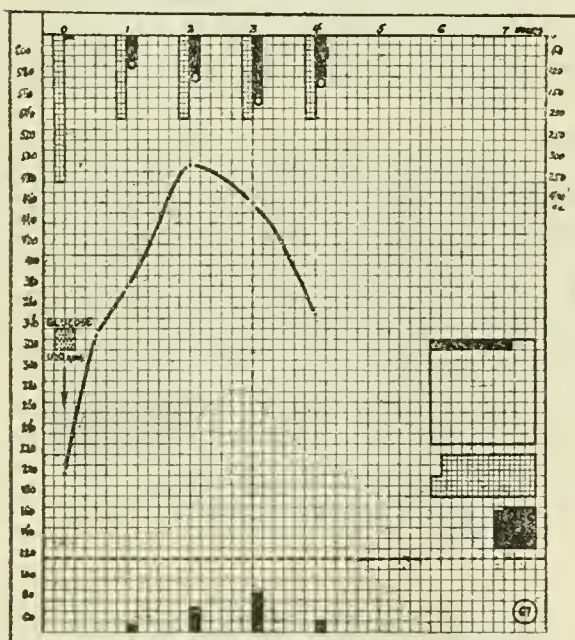
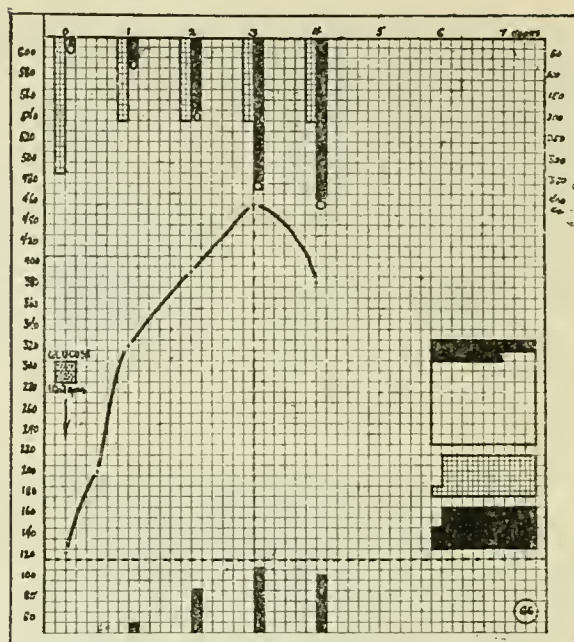
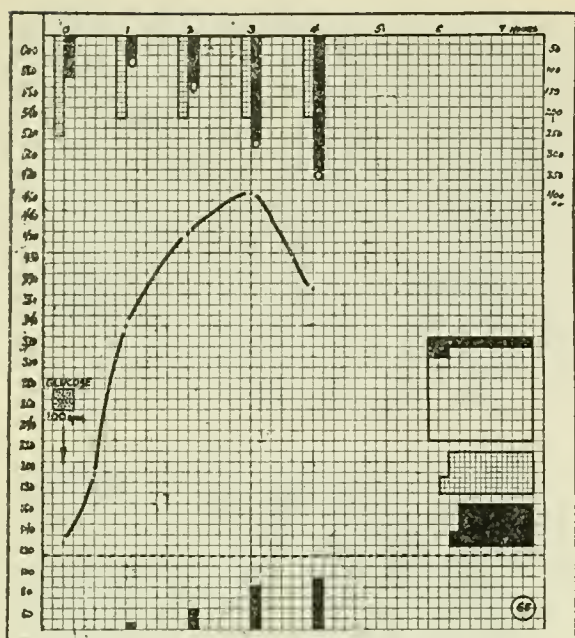




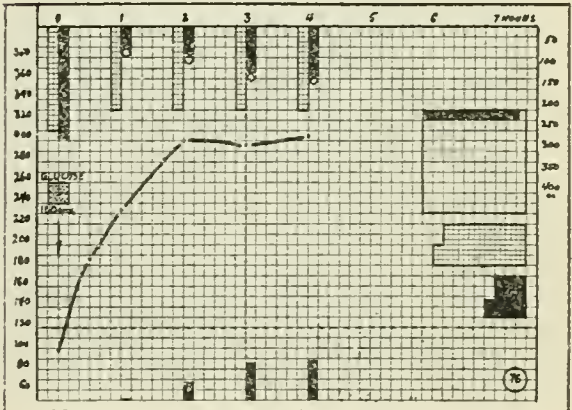
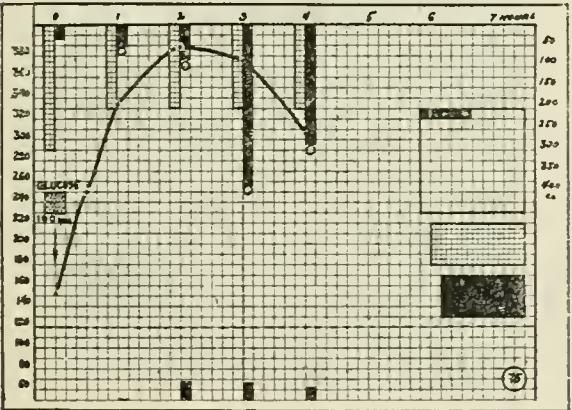
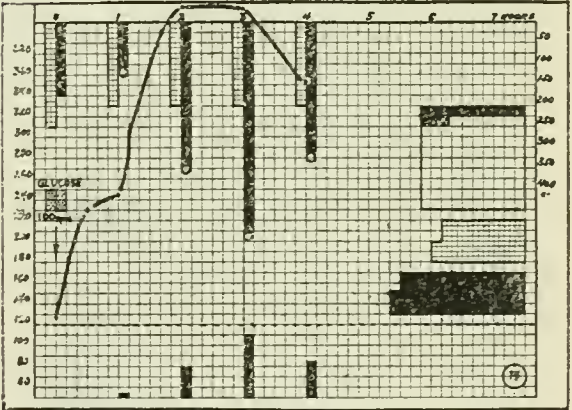
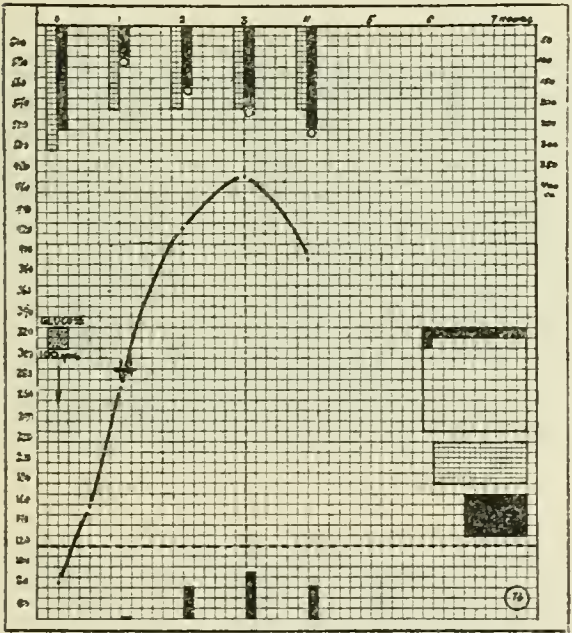
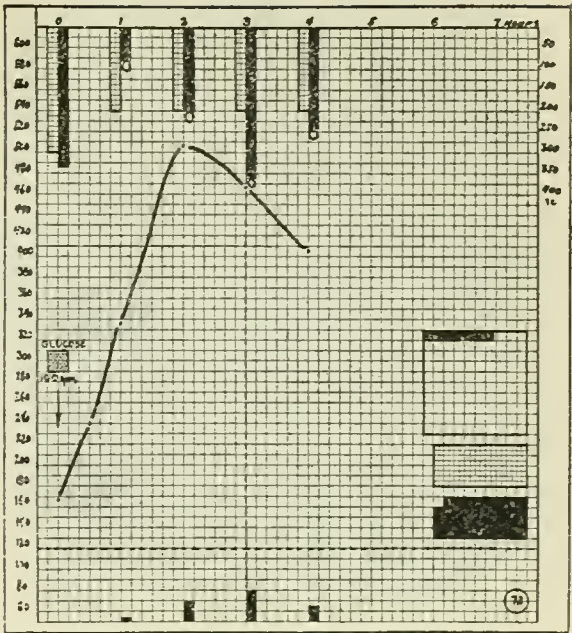
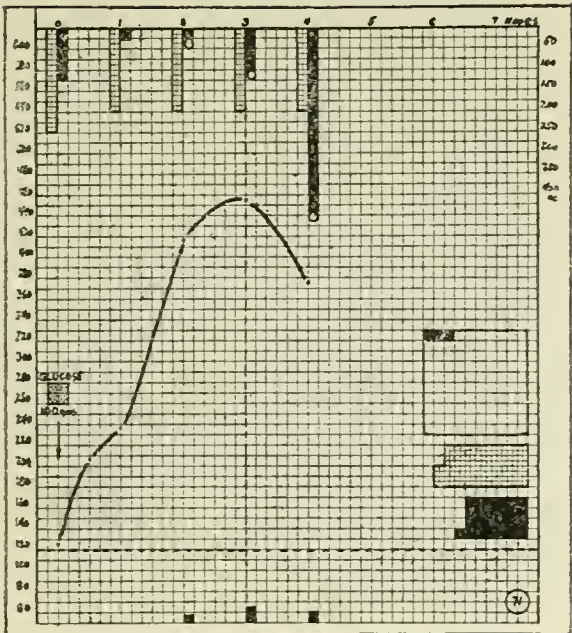




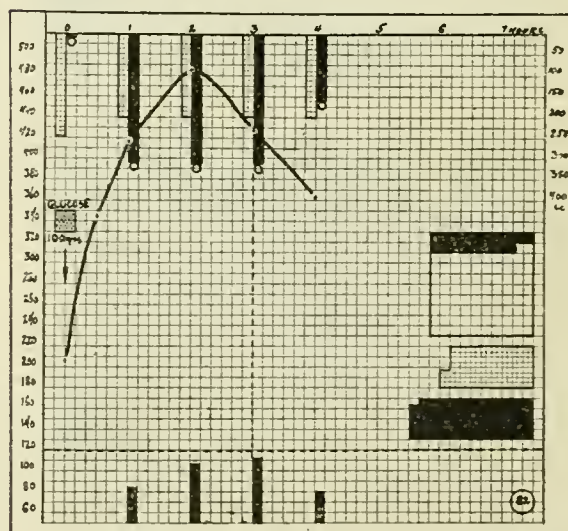
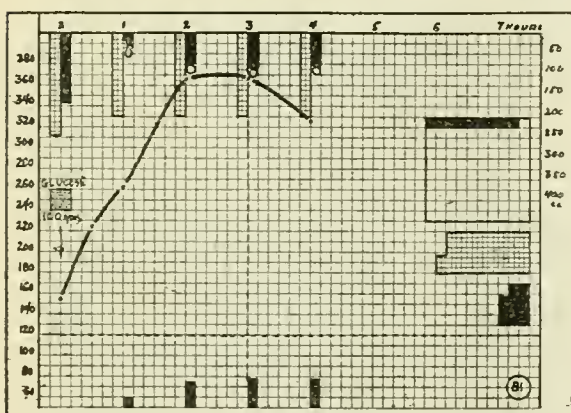
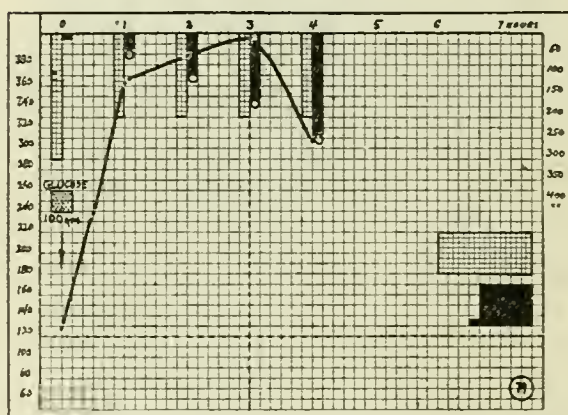
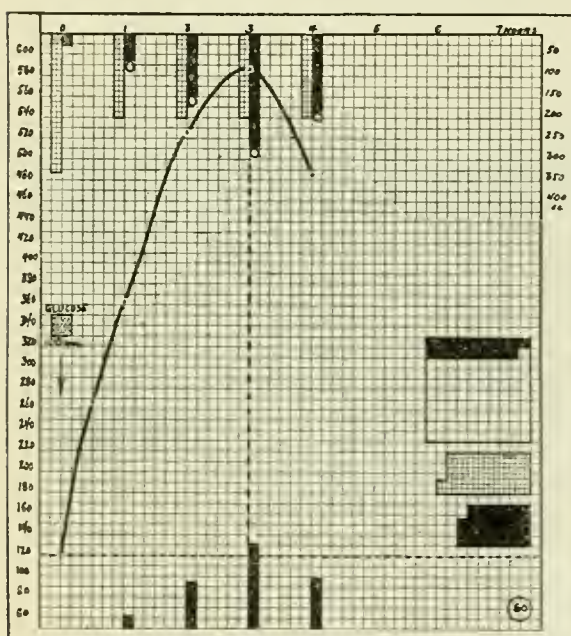
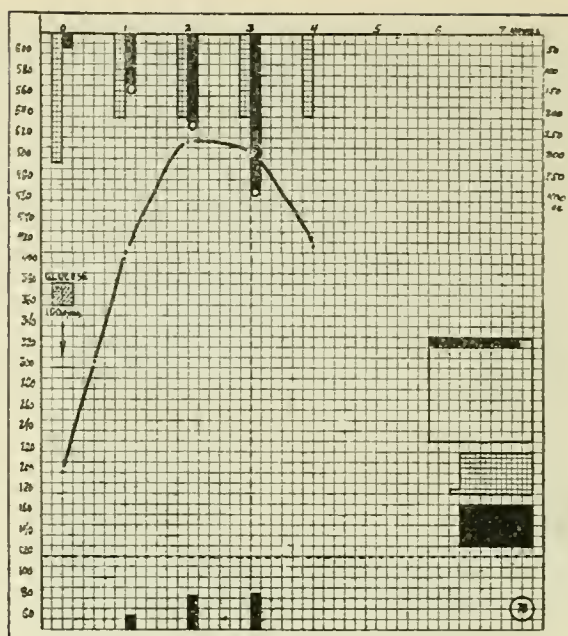
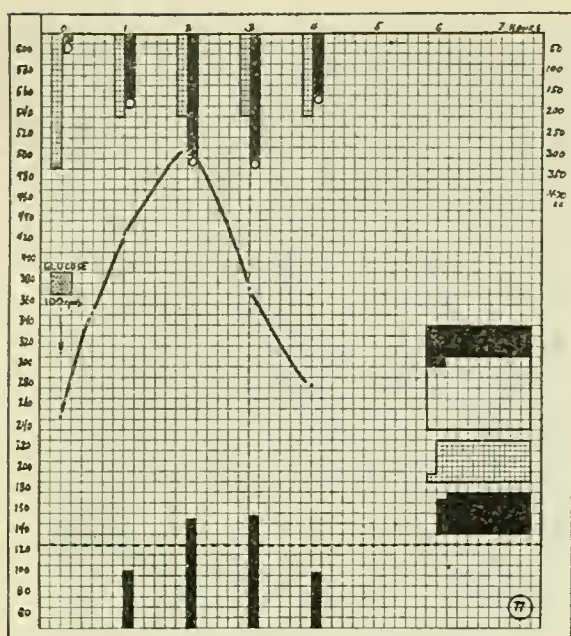




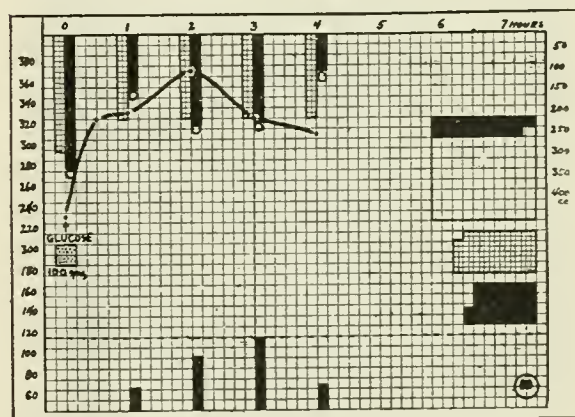
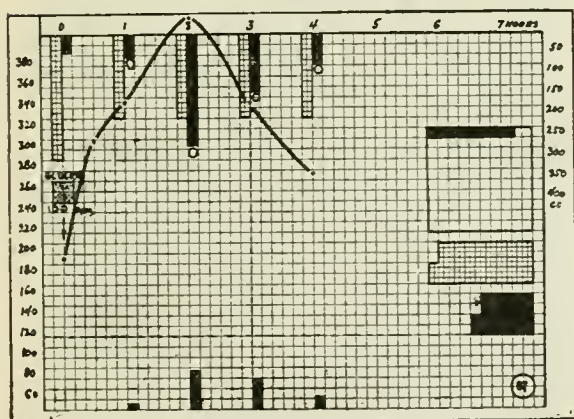
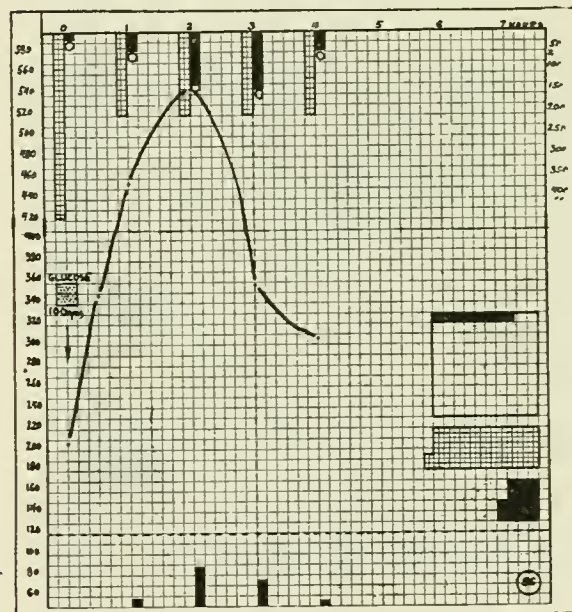
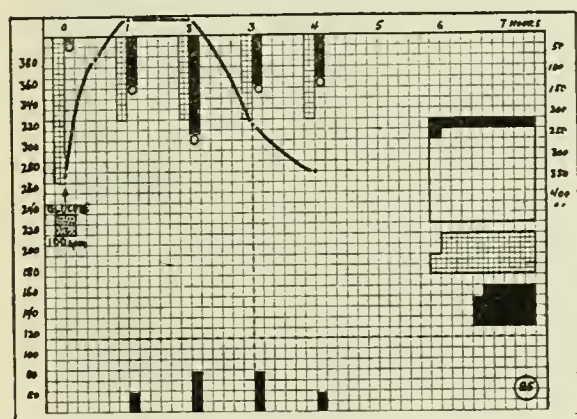
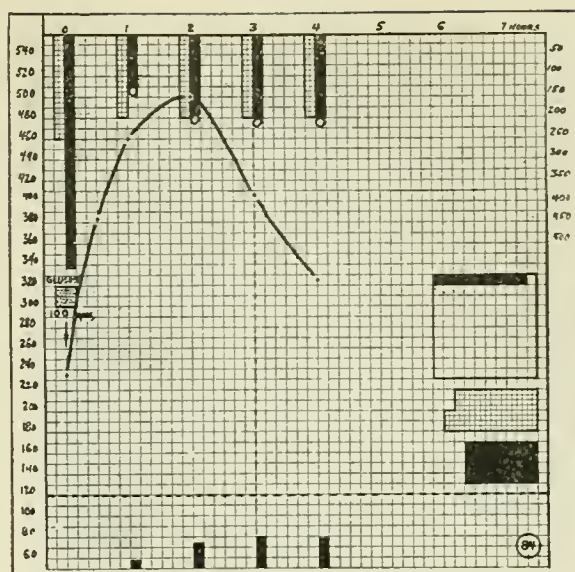
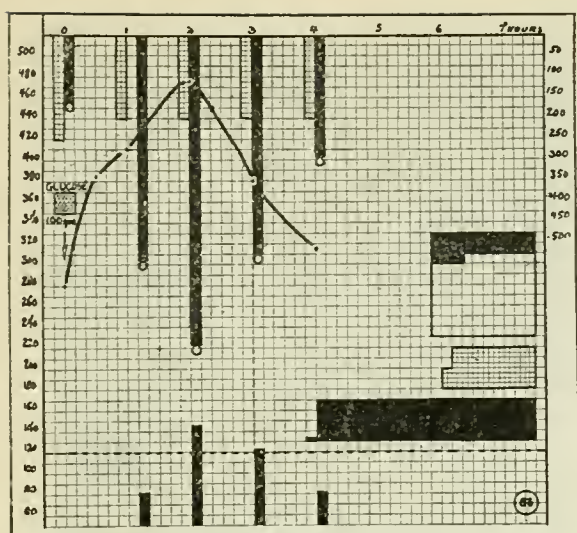




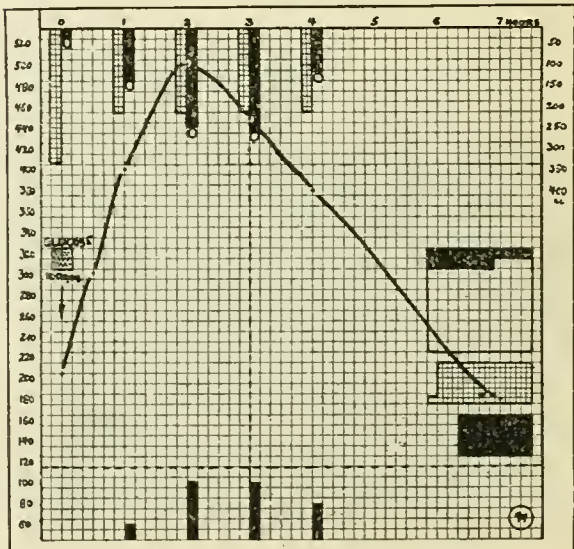
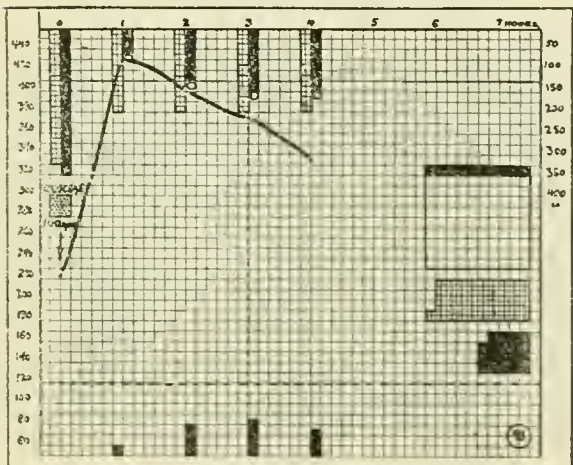
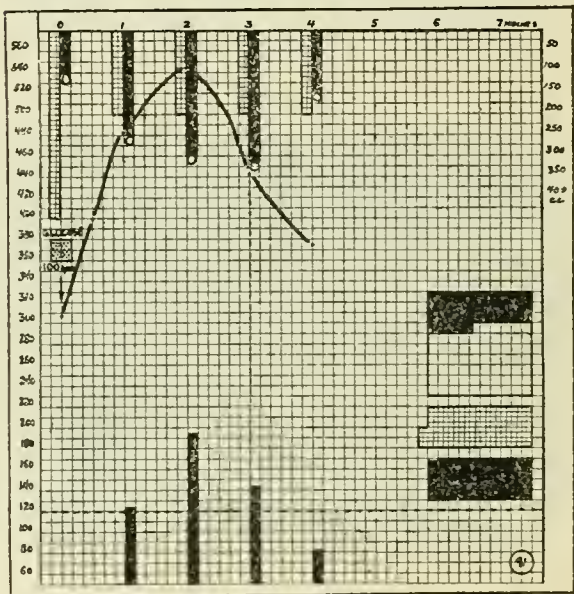
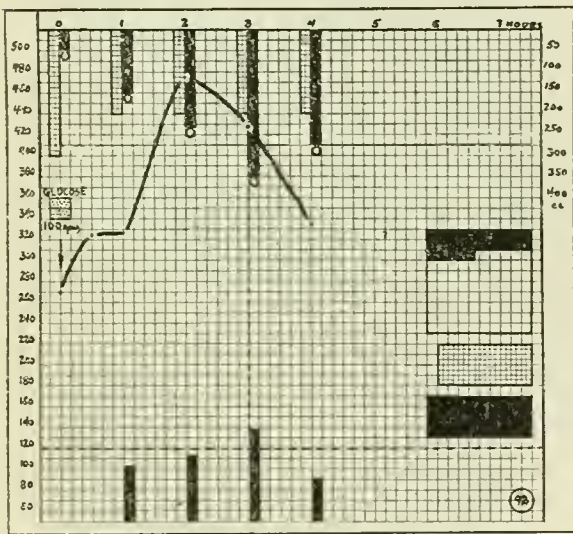
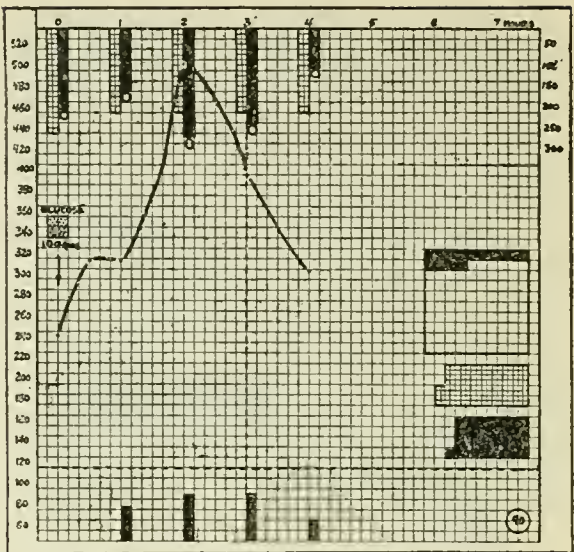
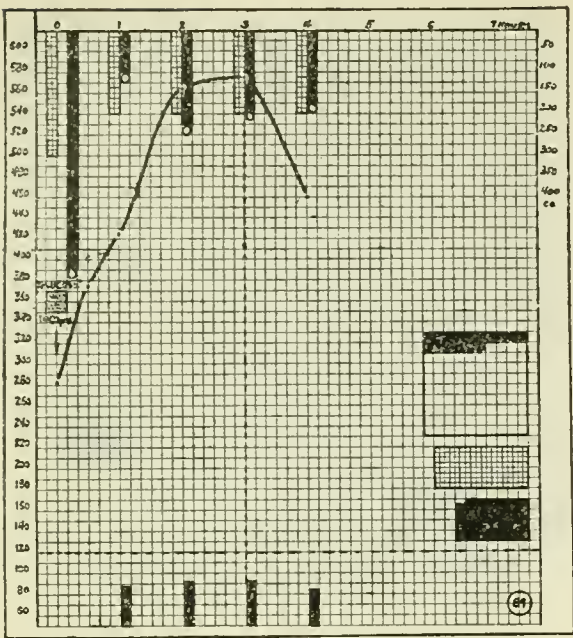




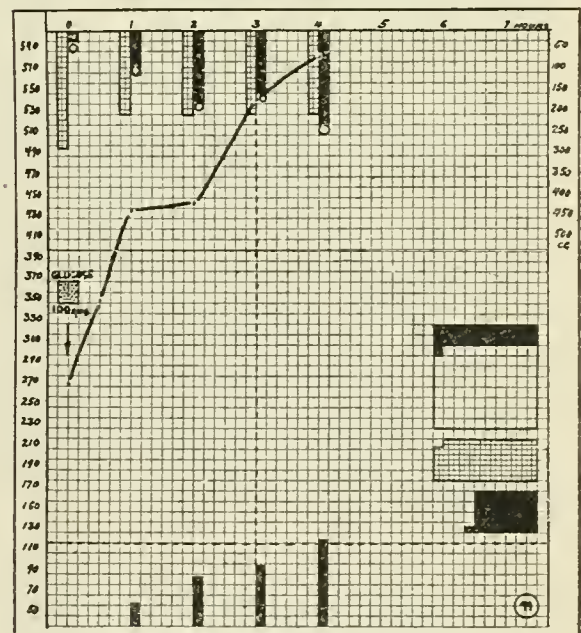
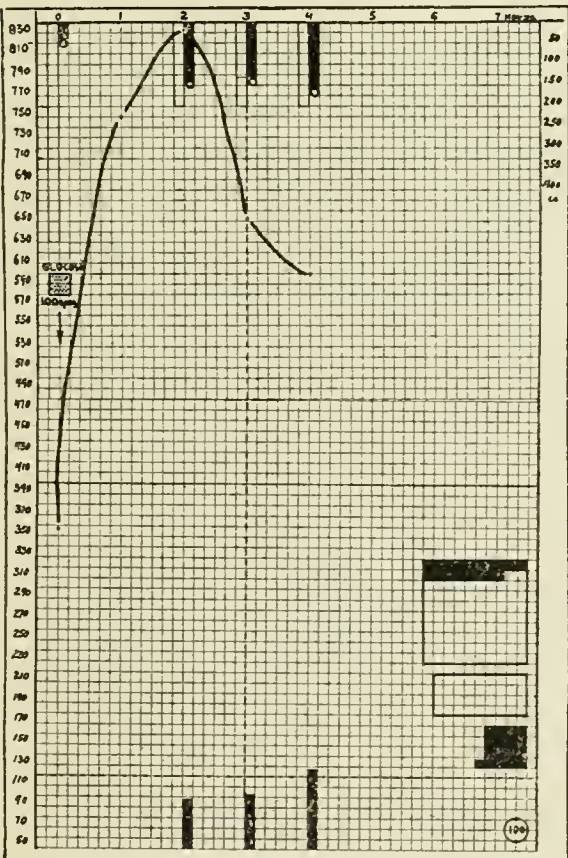
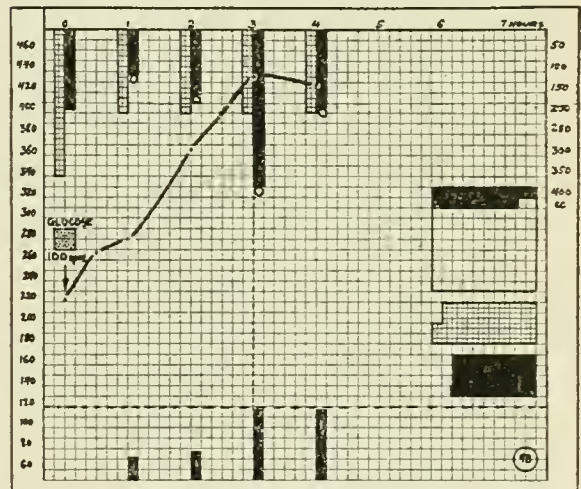
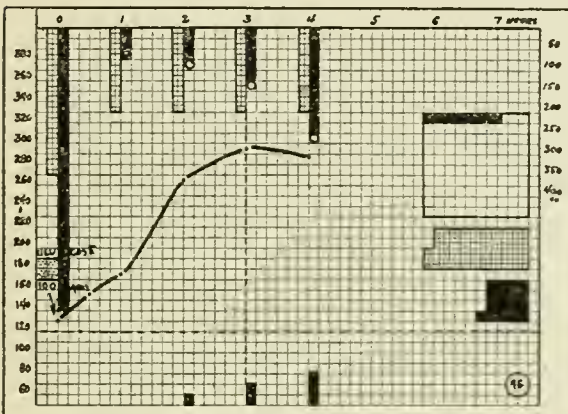
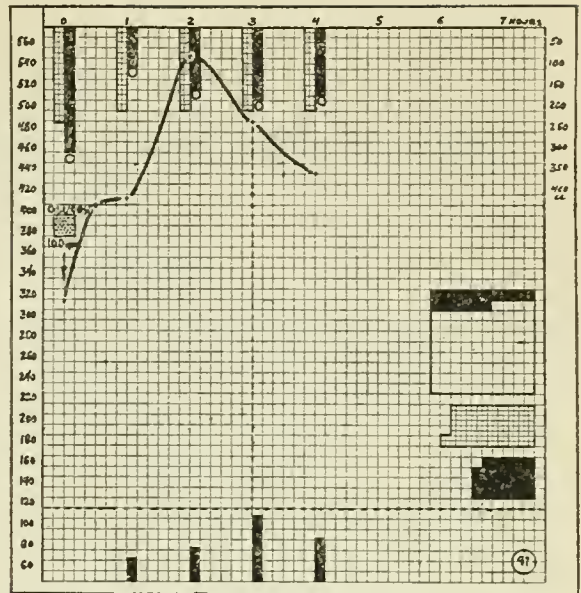
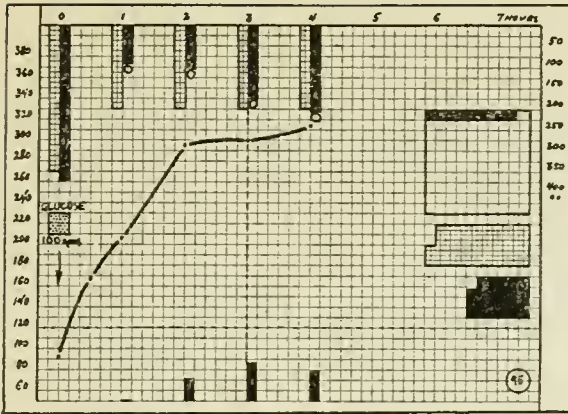














## THE URINE SUGAR AND ITS RELATION TO THE BLOOD SUGAR

By H. F. HÖST, Kristiania

The occurrence of sugar in the urine in pathological conditions (diabetes) was demonstrated by Dobson<sup>1</sup> as early as 1775, but not until the middle of the last century was it asserted by various observers that it might be present in normal urine.

Among the earliest investigations may be mentioned those of Brücke,<sup>2</sup> Bence-Jones<sup>3</sup> and Abel.<sup>4</sup> It is true that Frerichs<sup>5</sup> asserted in 1884 that normal persons even after taking large quantities of sugar did not excrete a trace, but in Scandinavia, Worm Müller,<sup>6</sup> as well as Nylander,<sup>7</sup> found that in a large percentage of healthy persons reducing and fermentable substances were present in the urine. Pflüger<sup>8</sup> was unable to confirm this and thought that the cause of the variable results must be differences in the diet of Scandinavia and Germany, an opinion which he considered was strengthened by the information "dass wegen der Kälte dort grosse Quantitäten stark gezucherten heissen Punsches getrunken werden . . ."

The investigations of Baumann and Wedenski,<sup>9</sup> and especially Baisch<sup>10, 12</sup> are of importance regarding the question as to whether glucose is a normal constituent of urine. Baumann had shown that when urine containing sugar is shaken with benzoyl chloride and caustic soda, a benzoyl-glucose compound separates out. On this principle Baisch worked out a method with which he was able to isolate a substance from urine that gave an osazone with phenylhydrazin, having the same melting point and N content as glucosazone. From these experiments of Baisch there is a widespread opinion that glucose is a normal constituent of urine.

Even the first workers in this field tried to get some idea of the *amount* of sugar in normal urine. By adding a weighed quantity of glucose to urine which had been fermented, Worm Müller<sup>6</sup> found that the limit of sensitiveness of his reaction was 0.25 pro mille glucose in urine, and with this to go upon he considered he could conclude from his experiments that normal urine contained up to 0.5-1 pro mille sugar. With a considerably improved, but, at the same time, more complicated technique, Schöndorff<sup>13</sup> in 1908 found 0.105-0.274 pro mille sugar in the urine of healthy persons. Even Schöndorff's method, however, was not really reliable and therefore many different workers (Cole,<sup>14</sup> Folin,<sup>15</sup> Myers<sup>16</sup>) attempted to elaborate new ways of determining the normal amount of sugar in the urine.

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\*These investigations were started at "The Post Graduate Hospital" [Prof. Myers], New York, 1919, and continued at "Rikshospitalet" [Prof. Laache], Kristiania, Norway.



In 1918, Benedict and Osterberg<sup>17</sup> described a method, the principle of which was to estimate the reducing power of the urine before and after fermentation, when creatinin and other reducing bodies (except carbohydrates) had been previously precipitated with Patein's fluid. The substances, the amount of which is determined before fermentation, the authors call "total sugar" in contradistinction to the "fermentable sugar" which is estimated as the difference between the total sugar and the reducing but non-fermentable substances. By this method, Benedict and his pupils investigated the sugar excretion in two normal dogs,<sup>18</sup> and in two healthy men.<sup>19</sup> From their investigations they conclude, on the whole, the following:

The reducing fermentable substances in urine consist entirely, or for the most part, of glucose, while the reducing non-fermentable bodies which are left in the urine after precipitation with Patein's fluid are probably sugars or closely allied products that form a stage in the digestion and absorption of glucose. On a constant diet the amount of "total sugar" in 24 hours is relatively constant. The sugar excretion is quite independent of the volume of the urine, but is greatly dependent upon the taking of food. It is greater with a diet containing carbohydrate than without. If small quantities of glucose (20 gm.) are taken with a meal the sugar excretion is much greater than when the same amount of glucose is given on a fasting stomach, which points to the fact that the external function of the pancreas (i. e., its digestive work) depresses the gland's internal function (regulation of carbohydrate metabolism). There is no absolute tolerance for sugar and the difference between the healthy and the diabetic organism is a purely quantitative one. In two healthy men on a mixed diet a total sugar excretion of about 1 gm. and 0.6-0.7 gm., respectively, in the 24 hours was found. On a diet containing an excess of fat and protein, the excretion was less, while on a diet rich in carbohydrate it was greater. The fermentable fraction of the total sugar constituted rather less than half of the latter. As clinical glycosuria must be looked upon as a purely quantitative augmentation of the physiological sugar excretion, Benedict substitutes the term "glycuresis" for glycosuria, and he maintains that the carbohydrate consumed by a person ought to be reduced, provided that the glycuresis exceeds 1.5 gm. (total sugar) in the 24 hours, because this proves that the individual's carbohydrate tolerance is diminished.

The results and conclusions of Benedict and his pupils are rather remarkable and, if they are correct, would be of great significance in our understanding of carbohydrate metabolism. In spite of this, Benedict's method has been employed to a relatively small extent. Beeler and Fitz,<sup>20</sup> who determined the total sugar in patients with adiposity, Kast, Wardell, and Myers,<sup>21</sup> and also Neuwirth<sup>22</sup> have all confirmed Benedict's finding of total sugar and fermentable sugar, but have not adopted his theoretical considerations. On the other hand, Schaffer and Hartman,<sup>23</sup> who used a modification of Benedict's method, did not succeed in producing fermentation in normal urine, and they therefore deny that the carbohydrate occurring in normal urine can be glucose.

Folin and Berglund<sup>24</sup> raise the objection against Benedict's technique that it is doubtful whether the fermentation of very small quantities of sugar can be used as a reliable quantitative method. They employ, therefore, a new method,<sup>25</sup> the principle of which is to estimate the reducing power of the urine after the protein, uric acid, creatin, and creatinin have been precipitated. On the results of investigations with this method, Folin and Berglund maintain that it is doubtful whether the normal urine sugar is glucose, and that glycosuria only occurs after meals containing carbohydrate and is due to the absorption and excretion of foreign, "unusable" carbohydrates found in grain, greenstuffs, and fruit.

The *relation of urine sugar to blood sugar* was demonstrated by Claude Bernard<sup>26</sup> as early as 1878, by proving that when the blood sugar rises to a certain height glycosuria appears. It was, however, Ivar Bang's<sup>27</sup> introduction of the micro-methods into medicine that first made possible a more thorough study of this condition. With Bang's method of blood sugar estimation, Jacobsen<sup>28</sup> examined 27 healthy persons, a number of cases of glycosuria, and some diabetics after the administration of glucose and starch. The examinations which were made at intervals of 15-30 minutes after taking 100 gm. glucose and 100 gm. starch (167 gm. white bread) showed that the blood sugar in healthy individuals rose to 110-180 mg. per 100 gm. blood. The maximum rise was found, as a rule, in 30 minutes, but displayed variations of from 15 minutes to 1 hour. A number of the persons investigated got glycosuria, both after glucose and after starch, others only after glucose, but the majority, after neither glucose nor starch. The urine was examined "at relatively short intervals when it could be done." After 50 gm. glucose the blood sugar curve exactly resembled that obtained after 100 gm. After the latter dose the blood sugar concentration returned to about its original value in  $\frac{3}{4}$ -2½ hours' time. With blood sugar rises less than 150 there was hardly ever glycosuria; between 150 and 180 it was present in some cases but not in others, and with concentrations greater than 180 there was nearly always glycosuria. In fasting diabetics hyperglycemia was regularly found and the blood sugar rise after carbohydrates was much greater and lasted longer than in normal persons. Fat and carbohydrate produced neither a blood sugar rise nor glycosuria in diabetics or sound individuals.

In recent years numerous works have been published dealing with the clinical glycosurias and the blood sugar after the administration of carbohydrates, especially glucose, per os,—the so-called tolerance tests. From considerations of space only a few will be mentioned. A number of the investigations into glycosuria give, on the whole, the same results as Jacobsen's, while others seem to confirm Frerich's contention that glucose can be given by the mouth in unlimited amount in healthy persons without producing glycosuria (Taylor and Hulton,<sup>29</sup> Folin and Berglund<sup>24</sup>).

There is, again, difference of opinion about the extent of the blood sugar rise after administration of glucose. Thus it is asserted in America (Hamman and Hirschman,<sup>30</sup> Folin and Berglund<sup>24</sup>) that the blood sugar



risers found for example by Jacobsen,<sup>28</sup> and later by Hagedorn,<sup>32</sup> are considerably higher than what they get in that country, a fact which Berglund<sup>24</sup> thinks may possibly be explained on the ground that the Danish hyperglycemia may be of psychic origin, incited by the pain which arises when the blood sample is taken by a puncture in the finger! Hagedorn<sup>31</sup> has shown, however, that the blood sugar rise after administration of glucose is much greater in the capillary blood than in venous blood, and as the Americans used venous blood, while the Scandinavian investigators employed capillary blood, the discrepancy is probably partly due to the different methods.

Since the blood sugar rise in the tolerance tests may vary markedly in both height and duration, Hagedorn<sup>32</sup> has tried to find, in the so-called assimilation value, a reliable measure of the blood sugar rise. The assimilation value, however, has not gained recognition in Denmark, where Faber<sup>33</sup> and Fridericia<sup>33</sup> hold that the duration of the blood sugar rise is a better measure.

While some investigators like Engstrand,<sup>34</sup> Pickering,<sup>35</sup> and others are of the opinion that the so-called renal threshold for glucose rises in diabetics with the duration of the disease, Faber and Norgaard<sup>36</sup> contend that it is constant in the individual patient, but varies greatly (90-190 mg. per 100 cc. blood) from one to another. Hagedorn<sup>32</sup> has on the whole confirmed Faber and Norgaard's results and also found that the position of the threshold, in healthy persons as well, is an individual characteristic.

Besides Benedict, only Folin and Berglund<sup>24</sup> have taken a stand with regard to the relation between glycemia and *physiological sugar excretion*. In opposition to Benedict, they maintain that the quantity of excreted sugar is independent of the blood sugar rise when it does not exceed the renal threshold.

### *The Author's Investigations* *Introduction*

As will be seen from the foregoing review it must be regarded as certain that normal urine contains small quantities of carbohydrates. Their nature, however, has not been accurately determined and the question whether glucose is a physiological constituent of the urine is especially open to debate.

The amount of carbohydrates in urine was estimated by various methods (Benedict and Osterberg,<sup>17</sup> Folin and Berglund,<sup>25</sup> Schaffer and Hartmann,<sup>23</sup>) the reliability of which the authors have only partially demonstrated. The investigations were made in relatively few individuals and only in healthy ones, and opinion is divided as to whether the various factors which influence the excretion are operative in them.

The degree of hyperglycemia and the occurrence of clinical glycosuria after taking carbohydrates, particularly glucose, are



the subject of great differences of opinion. This has undoubtedly many causes, one of which, the determination in capillary or venous blood has been discussed already. A priori, a second reason may be that some investigators have taken only a few blood samples while others have taken frequent ones for the tolerance tests. From numerous investigations into the course of the blood sugar curve after giving large amounts of glucose (50-100 gm.), we know that the blood sugar rises rather rapidly, attains a more or less sharp maximum, and then sinks more slowly to the normal level. Since the position of this maximum may vary extremely, as Jacobsen's investigations<sup>28</sup> have already proved, the blood samples must be taken at short intervals,—every quarter of an hour or preferably more frequently still,—if we wish to detect the actual apex of the blood sugar curve. When Folin and Berglund<sup>24</sup> and many others only take samples at intervals of  $\frac{1}{2}$ -1 hour and at variable intervals in different experiments, it is impossible for them to get even an approximate idea of the height of the blood sugar curve.

The same applies to the large majority of investigations into glycosuria after administration of glucose by the mouth,—the tolerance tests, which have been employed in the diagnosis of doubtful cases of diabetes,—and to a number of diseases of the endocrine glands (thyroid, suprarenals, pituitary). The use of different methods for demonstrating sugar in the urine and lack of uniformity in the estimation of the course of the reaction are certainly of less importance in this case. Of far greater consequence is undoubtedly the number of times the person investigated has passed urine. In tolerance tests it is extremely important to be able to detect very slight glycosuria, which is only present,—as a rule in healthy persons,—for the very short time during which the blood sugar concentration is higher than the threshold. If we happen to collect the urine during this interval a qualitative sugar reaction will give a positive result, but if this sample is mixed with urine collected before or after, as is done when the individual passes urine only once every hour, the sugar concentration in the urine may decrease so much that the qualitative test gives a negative result. Many of the authors who have made tolerance tests have let their experimental subjects pass urine much too infrequently. Folin and Berglund,<sup>24</sup> as well as Motzfeldt,<sup>27</sup> for example, collected the urine every hour, while

Taylor and Hulton<sup>29</sup> have even used a 24 hours' sample to determine whether glycosuria occurs after a single large quantity of glucose.

The amount of diuresis is also, of course, important as a large diuresis might prevent the detection of slight glycosuria, a difficulty which Faber and Norgaard<sup>36</sup> tried to surmount by evaporating the urine. By this means they introduce a new factor—the evaporation—the effect of which on traces of sugar in the urine they have not examined, as far as one can ascertain. The first desideratum to obtain comparable results in tolerance tests is therefore a uniform technique for collection of the urine and detection of the sugar, and to be as certain as possible of demonstrating incipient glycosuria, the urine must be collected at the shortest possible intervals. But the power of frequent micturition is limited and varies greatly from person to person, and qualitative methods are unsuitable especially for the demonstration of incipient glycosuria of a very slight increase of physiological glycosuria. For an accurate study of the conditions governing the excretion of glucose, as Hagedorn<sup>32</sup> also points out, quantitative estimations of the sugar in the urine are necessary.

With these considerations in mind the plan of the present work has been:

- I. To inquire whether Benedict and Osterberg's method for the quantitative estimation of sugar in the urine is reliable, and possibly also,
- II. To determine in healthy persons,
  - a. The amount of sugar in the 24 hours' urine.
  - b. The relation of the sugar in the urine to:
    1. The absorption of water (diuresis).
    2. Acids.
    3. Alkalis.
    4. Mixed diet.
    5. Single articles of food.
    6. The blood sugar.
- III. To investigate the relation between the urine sugar and the blood sugar in persons with chronic or intermittent glycosuria,—“transition cases.”

IV. To investigate the excretion of urine sugar in sugar-free diabetics in the 24 hours' urine and after different meals, and the administration of glucose.

V. To try to throw light on the question whether glucose is a normal constituent of urine.

#### Methods

The methods which have been employed in the present work are Benedict and Osterberg's method for the quantitative estimation of urine sugar, Hagedorn and Norman Jensen's method<sup>38</sup> for the quantitative estimation of blood sugar, and various qualitative methods for the determination of sugar in urine, namely, Benedict's, Almén's, and the phenylhydrazin reaction.

With Benedict and Osterberg's method control determinations were made first of the total sugar, and then fermentation in normal urine was investigated.

To a portion of urine from a healthy person a weighed quantity of glucose was added. The total sugar was estimated before and after the addition of the sugar.

#### Experiment No. 1.

Urines	Total sugar pro mille	Glucose added pro mille	Total sugar found calculated	
1	0.24	0.4	0.50	0.64
2	0.44	0.4	0.70	0.84
3	0.52	0.5	0.86	1.02
4	0.47	0.5	0.81	0.97

Many experiments showed, as did these four, that the method gave too low values. In order further to investigate where the error lay, 1 gm. glucose was dissolved in 100 cc. urine and it was then treated by Benedict and Osterberg's method. The filtrate after precipitation with the nitrate of mercury solution was found to contain 0.5% sugar with the polarimeter. No sugar had, therefore, been lost in the precipitating process. The filtrate was further treated with HCl and Zn. The filtrate from this again showed 0.5% sugar with the polarimeter. Picrate solution and soda were added in the prescribed way to the filtrate, which was then heated in a water bath. As a standard, 1 per thousand glucose solution was employed. After being in the water bath.

#### Experiment No. 2.

In 10 minutes were found 1.6 pro mil. instead of 5 pro mil.									
" 15	"	"	"	2.3	"	"	"	5	"
" 20	"	"	"	3.6	"	"	"	5	"

It was clear from these experiments that the error in the method did not occur until after the treatment with the picrate solution and the heating in the water bath. In order to investigate the effect of the hydrogen ion concentration on the course of the reaction, 5 drops of con-



centrated HCl were added to 5 cc. of the filtrate after the Zn-HCl precipitation, which was alkaline, after which the reaction became slightly alkaline, whereupon picrate solution was again added and the whole heated in the water bath.

After remaining in the water bath for 10 minutes, the amount of sugar present was 3.2 pro mille. From these and many similar experiments it appeared that an excess of alkali in the filtrate after the Zn-HCl precipitation limited the intensity of the color produced (formation of picramic acid), and also that a prolonged stay in the water bath to a certain extent counteracted the excess of alkali. The experiments showed, therefore, that the hydrogen ion concentration was of the greatest importance in the method, which, moreover, Benedict and Osterberg state without indicating further how the correct reaction is to be secured. A fresh series of experiments were, therefore, made to try to unravel the conditions under which the method would give accurate results.

On investigating a series of urines, it was found that about 4 gm. sodium bicarbonate had to be added to 20 cc. urine + 20 cc. mercury solution to give it just a distinct alkaline reaction. On employing these relative amounts the added quantity of glucose was recovered in the subsequent control analyses in the majority of cases, but the heating in the water bath had to be continued for 20-25 minutes. If more sodium bicarbonate was used less sugar was always left over, although this could be compensated for by adding concentrated HCl drop by drop to the filtrate after treatment with Zn-HCl until a weak alkaline or neutral reaction was produced. The result of numerous experiments thus was that 4 gm. of sodium bicarbonate was added to 20 cc. urine + 20 cc. mercury solution,\* and shaken until the frothing had ceased and the mixture reacted distinctly alkaline. Only in rare cases, for example after HCl had been given *per os*, was it essential to use more sodium bicarbonate. After filtration and treatment of the filtrate from the HCl and Zn, about 5 cc. was taken, to which concentrated HCl was added drop by drop until a weakly alkaline or neutral reaction was obtained. If this made the reaction acid, some of the original filtrate was added till it again became neutral or faintly alkaline. By this means and by heating in the water bath for 20 minutes instead of 10 minutes, it was always possible to detect a quantity of glucose added to urine within an error of 10-15%.

After thus having made sure that the method gave correct values on the addition of varying amounts of sugar to urine, a series of experiments on fermentation in urine was undertaken. Benedict in his paper only states that the urine must ferment 18-20 hours at 35°-38°, as mentioned above.

There are already a number of investigations in the literature dealing with the fermentation of small amounts of sugar. Thus Seegen<sup>39</sup> reports that a 0.6-0.3% watery solution of glucose, to which is added tartaric acid and yeast, after 48 hours contains 0.02-0.03% of a reducing substance,

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\* As the opportunity of analyzing so much as 20 cc. urine did not often present itself, and the reagents for these analyses are very dear, an aliquot part of the amounts named was usually employed, e. g. 10 cc. urine + 10 cc. mercury solution + 2 gm. bicarbonate.

and von Lippman<sup>40</sup> even asserts that a 0.1% sugar solution practically does not ferment at all. On the other hand, Ege and Rasmussen<sup>41</sup> find that 1 pro mille glucose dissolved in 0.9% NaCl to which 1% yeast is added contains 0.01 pro mille sugar after 24 hours. They further maintain that the power of fermentation can vary in different samples of yeast, but it is constant for the same sample, and also that the amount of yeast is important. Accurate investigations into the fermentation of small quantities of glucose in urine, as far as I can ascertain, do not exist.

The experiments of Neuberg<sup>42</sup> and Mayer<sup>43</sup> are of importance in this connection. They show that a pure solution of yeast contains and forms during fermentation not inconsiderable amounts of reducing substances, such as pentoses, purins, etc. It was, therefore, first necessary to investigate whether yeast as such formed bodies which reduced picric acid solution, and afterwards carry out some experiments on fermentation in urine.

In Benedict and Osterberg's colorimetric method the picric acid solution employed possesses a color of its own, which is of no importance in the estimation of sugar solutions because the standard solution and the solution whose sugar concentration is to be measured is added to it, and the picramic acid formed obscures the color provided that the glucose concentration is not very low. But this color must be taken into account when deciding whether a solution contains very small amounts of glucose or whether it is free from sugar.

In order to elucidate this point further, 4 cc. of picric acid solution was mixed with 4 cc. distilled water and 1 cc. soda solution, and then heated in the water bath as usual, using as a standard a watery solution containing 0.10 mg. glucose. From this and many similar experiments it was discovered that the color due to picric acid itself had a value equivalent to about 0.05 pro mille of sugar.

A number of experiments were next made on the formation of reducing bodies by ordinary commercial yeast.

#### *Experiment No. 3.*

5 gm. of yeast was added to 100 cc. of Sørensen's phosphate mixture with PH 6.64 and the mixture put in thermostat at 37°.

After 24 hours were found 0.08 pro mil. "sugar."

" 48 " " " " 0.07 " " "

#### *Experiment No. 4.*

5 gm. of yeast was added to 100 cc. of distilled water. After staying in incubator at 37°.

For 24 hours the mixture contained 0.06 pro mil. "sugar."

" 48 " " " " 0.07 " " "

Many experiments with different yeast samples gave the same result.

As 0.05 pro mille must be allowed for the color of the liquid, the yeast forms about 0.01-0.02 pro mille reducing substances with the method employed here. We may entirely neglect these very small amounts in the following investigations, as the error they give rise to falls within the limits of the experimental error.

Experiments on the fermentation of small amounts of sugar were now undertaken.

*Experiment No. 5.*

100 mg. glucose and 5 gm. yeast were mixed with 100 cc. distilled water and put in incubator at 37°.

After 24 hours the mixture contained 0.17 pro mille.

"	48	"	"	"	"	0.05	"	"
"	72	"	"	"	"	0.05	"	"

The fermentation in watery solution was, as will be seen, finished after 48 hours.

*Experiment No. 6.*

To phosphate mixtures with different hydrogen ion concentration were added glucose to 1 pro mille and yeast to 5 per cent., after which the mixtures were put in incubator at 37°.

No. 1	PH	7.38
" 2	"	6.64
" 3	"	5.90
" 4	"	4.94

After 24 hours all the samples contained 0.06 pro mille "sugar," consequently the fermentation was complete.

The fermentation in urines from healthy persons was then studied. Different samples of the same urine were added different amounts of yeast and of glucose.

*Experiment No. 7.*

Urine from healthy person, spec. gravity 1017, contained no albumin. Total sugar 1.08 pro mille. From this urine were measured portions, each containing 100 cc., to which were added different amounts of yeast and of glucose as follows:

No. 1, 100 cc. of urine + 100 mg. of glucose + 2 gm. of yeast.									
" 2,	"	"	"	"	+	0	"	"	"
" 3,	"	"	"	"	+	0	"	"	"
" 4,	"	"	"	"	+	0	"	"	"
" 5,	"	"	"	"	+	200	"	"	"
" 6,	"	"	"	"	+	300	"	"	"

After being incubated the samples had the following reduction values:

No. 1	24 hr.	48 hr.	72 hr.
	lost	0.63 pro mil.	0.65 pro mil.
" 2	0.97 pro mil.	0.59 " "	0.60 " "
" 3	0.90 " "	0.58 " "	0.57 " "
" 4	0.90 " "	0.59 " "	0.58 " "
" 5	0.94 " "	0.56 " "	0.57 " "
" 6	0.94 " "	0.57 " "	0.60 " "



*Experiment No. 8.*

6 urines from different persons:

Urine No. 1 from convalescent, spec. gravity 1018, no alb. or Benedict\*

"	"	2	"	"	"	"	1019,	"	"	"	"
"	"	3	the same as No. 2								
"	"	4	from a convalescent		"	"	1024,	"	"	"	"
"	"	5	from a diabetic		"	"	1012,	no albumin			
							sugar 1 per cent.				

All the samples were added yeast to make 5 per cent. and glucose to make 1 pro mille, with the exception of urine No. 3, to which was added no glucose.

Urine No.		Total sugar		After being incubated for	
		before fermentation and before glucose was added		48 hr.	72 hr.
1		0.63 pro mil.		0.55 pro mil.	0.60 pro mil.
"	2	1.05	" "	0.60	0.58 " "
"	3	1.05	" "	0.60	0.61 " "
"	4	1.10	" "	0.65	0.66 " "
"	5	10.0	" "	0.75	0.73 " "
"	6			0.68	0.70 " "

Both these series of experiments show that the amounts of sugar and yeast within rather wide limits do not affect the time required for the fermentation to reach its end-point. They show, in other words, that the fermentation proceeds very rapidly with relatively large quantities of sugar in the urine, but slowly when the sugar concentration falls. The experiments further prove that the fermentation is not finished after 24 hours, but is at an end in all cases after 48 hours, and also that it is not essential to add glucose to urine to start fermentation going. The introduction of small amounts of glucose into the urine before fermentation is started, however—as Benedict and Osterberg mention—serves to control the activity of the yeast, and in all the following experiments about 1 pro mille glucose is added to the samples of urine. About 1 gm. of yeast is added to about 20 cc. urine, that is, about 5% yeast.

Below are tabulated the course of fermentation in different urines from healthy persons and from convalescents.

\* By "Benedict" is meant Benedict's qualitative sugar reaction.

*Experiment No. 9.*

		Before fermentation	After being incubated for			
			24 hr.	48 hr.	72 hr.	96 hr.
Urine No. 1		0.30 pro mil.	0.15	0.15		
" "	2	0.60 " "	0.60	0.45	0.45	
" "	3	1.04 " "		0.48	0.49	
" "	4	0.90 " "	0.58	0.40	0.35	0.37
" "	5	0.90 " "	0.90	0.45	0.49	
" "	6	1.42 " "	1.02	0.64	0.60	
" "	7	0.92 " "	0.90	0.46	0.49	
" "	8	0.37 " "	0.30	0.23	0.22	
" "	9	0.95 " "	0.37	0.27	0.25	
" "	10	1.14 " "	0.60	0.44	0.50	
" "	11	0.78 " "	0.57	0.30	0.32	
" "	12	0.72 " "	0.42	0.24	0.26	

In two urines, incubated at 37°, the fermentation was studied for 5 days:

*Experiment No. 10.*

		Before fermentation	After being incubated		
			24 hr.	48 hr.	5 days
Urine No. 1		1.37 pro mil.	0.90	0.72	0.80
" "	2	1.0 " "		0.51	0.45

In addition the fermentation was studied in some urines from diabetics:

*Experiment No. 11.*

		Before fermentation	After being incubated for		
			24 hr.	48 hr.	72 hr.
Urine No. 1		2.4 pro mil.	0.33	0.24	0.22
" "	2	2.76 " "	1.08	0.65	0.60
" "	3	1.12 " "	0.29	0.30	
" "	4	1.43 " "	0.27	0.25	
" "	5	0.84 " "	0.23	0.25	0.26

All these experiments go to show that fermentation is usually not finished after 24 hours in the thermostat, but comes to an end after 48 hours. It frequently happened, however, that after remaining in the thermostat for a long time, 48 hours or more, a marked production of ammonia took place which prevented further fermentation, and the urine samples were, therefore, heated before adding the yeast. This partially inhibited the evolution of ammonia, but did not entirely stop it, since the yeast used was evidently infected with bacteria. As it seemed possible that fermentation would proceed farther if it could go on without the presence of ammonia, I obtained through the kindness of Dr. Geelmuyden a number of pure cultures of yeast ("Logos-Hefe, van Laer").

*Experiment No. 12.*

The yeast culture received was added under sterile conditions to urine which had previously been boiled for 5 minutes and cooled. At the same

time ordinary commercial yeast was added in the customary amount to another sample of the same urine. The total sugar before fermentation was 1.10 pro mille. After 48 hours, fermentation was at an end in the sample containing ordinary yeast. It then contained 0.58 pro mille reducing substances and was distinctly, though not strongly, ammoniacal. In the urine sample to which the yeast culture was added there was no production of ammonia even after 5 days, but fermentation none the less proceeded very slowly, though not until 5 days had passed did it show about the same concentration of reducing substances, 0.63 pro mille, as the first sample. The reason for this slower fermentation was undoubtedly, in part, that the amount of commercial yeast in this case was much greater than the amount of culture yeast. As the latter yeast had to be filtered sterile from its nutritive medium and washed, while various substances, as is known, are put with the commercial product for purposes of preservation, it was impossible to get an accurate idea of the amount of yeast in the two samples. Although the experiment was subject to the error that the quantity of yeast in the two samples was different, the outcome seemed undoubtedly to favor the view that the non-fermentable portion of the total sugar did not owe its existence to cessation of fermentation on account of ammonia production, but that the fermentation in urine under the conditions employed was actually complete.

As will be observed from the experiments the fermentation proceeded much more slowly in urine than in watery solution and in phosphate mixtures. The influence of two other factors, namely, the concentration and the hydrogen ion concentration, on the fermentation in urine was therefore investigated.

*Experiment No. 13.*

Urine from healthy persons, spec. gravity 1026.

In incubator				
	Total sugar	24 hr.	48 hr.	72 hr.
Urine	1.42	1.02	0.64	0.51
Urine, diluted with equal amount of water				

In incubator				
	Total sugar	24 hr.	48 hr.	72 hr.
	0.71	0.32	0.27	0.27

Urine from a diabetic, spec. gravity 1020.

In incubator			
	Total sugar	24 hr.	48 hr.
Urine	0.80	0.27	0.28
Urine + Water	0.40	0.15	0.14

Fermentation went rather more rapidly in the first diluted urine than in the undiluted urine, but the difference was not great, and in the second urine there was no difference in the rate. The molecular concentration thus does not seem to be of any great moment in the fermentation of urine.



The significance of the hydrogen ion concentration was investigated partly by fermenting samples of urine whose pH values were different, and partly by varying the pH value artificially by adding primary or secondary phosphate to different samples of the same urine and then fermenting them. The pH value was determined by the colorimetric method<sup>41</sup> which I have previously described.

*Experiment No. 14.*

		From diabetics				
		pH.	Before fermentation	After being incubated for 24 hr.	48 hr.	72 hr.
Urine	No. 1	5.58	0.63	0.27	0.30	
"	" 2	5.70	1.0	0.32	0.33	
"	" 3	5.70	0.51	0.30	0.23	0.22
"	" 4	6.0	0.84	0.23	0.25	
"	" 5	5.70	0.80	0.28	0.30	
"	" 6	5.6	0.91	0.46	0.45	
"	" 7	5.0	1.05	0.45	0.40	0.33
"	" 8	5.2	0.93	0.44	0.40	
		From healthy persons				
"	" 9	5.9	1.25	0.75	0.50	0.53
"	" 10	5.7	1.05	0.32	0.32	

*Experiment No. 15.*

In the following urines the hydrogen ion concentration was varied by addition of primary or secondary phosphates.

*Urine No. 1.* Spec. gravity 1023, contains no albumin; pH 5.9.

pH.	Sugar before		After being incubated for			
	fermentation		24 hr.	48 hr.	72 hr.	
4.90	1.40	pro mille	1.15	1.02	0.62	0.46
5.90	1.40	" "	0.97	0.50	0.40	0.39
6.46	1.40	" "	0.45	0.30	0.30	0.30
7.38	1.40	" "	0.66	0.43	0.46	0.30

*Urine No. 2.* Spec. gravity 1015, contains no albumin.

pH.	Sugar before		After being incubated for			
	fermentation		24 hr.	48 hr.	72 hr.	
4.90	0.78	pro mille	0.73	0.50	0.45	
6.23	0.78	" "	0.45	0.40	0.38	
6.60	0.78	" "	0.42	0.38		
7.14	0.78	" "	0.55	0.45	0.40	

*Urine No. 3.* Spec. gravity 1020, contains no albumin; pH. 5.0.

pH.	Sugar before		After being incubated for			
	fermentation		24 hr.	48 hr.	72 hr.	
5.0	1.20	pro mille	0.72	0.60	0.48	
6.60	1.20	" "	0.50	0.48	0.49	

As will be seen from the experiments the hydrogen ion concentration is of the greatest importance in the fermentation of urine, since the latter proceeds more slowly in weakly alkaline urine and in relatively strongly acid urine than when the urine is slightly acid. In the first experiments on fermentation a little tartaric acid was added to the urine as was done by Seegen<sup>38</sup> and others, but the fermentation then proceeded very slowly, often not being finished for 72-96 hours. But there was never any ammonia production in the urine in these experiments. The result of the experiments on the influence of the hydrogen ion concentration on fermentation make it likely that the added tartaric acid rendered the urine too acid, which was the cause of the very slow action that took place in the first experiments on fermentation in urine.

In two urines, 1 and 3 (see experiment 15), fermentation was not complete in 48 hours, but came to an end in 72 hours, while those adjusted to pH 6.46 and 6.60, respectively, by the addition of secondary phosphate showed complete fermentation in 48 hours. Moreover, the urines whose pH value was not varied artificially gave complete fermentation in 48 hours. The hydrogen ion concentrations, which were experimentally produced in the urines fell, however, within the limits found in urine. Consequently in the following experiments I have always sought to bring the pH value of the urine within the optimal zone for fermentation, that is between 5.5 and 6.8 by adding primary or secondary phosphate. This proved to be especially necessary after the administration of acid and bases by the mouth (see later).

It appears from the experiment that fermentation in urine, in spite of an optimal hydrogen ion concentration, proceeds much more slowly than in watery solution or phosphate mixture. The cause of this delay may be imagined to be one of two things. As glucose added to urine always ferments very quickly it is possible that the normal fermentable substance in urine is not glucose, but a closely related carbohydrate which ferments more slowly. But we may also suppose that substances are present in urine which inhibit fermentation. As already pointed out, this is the case when ammonia is produced in a certain quantity. These theories made it desirable to inquire whether fermentation would take place in the filtrate from the Zn-HCl precipitation, which has been freed from most of the urinary constituents. A series of experiments was, therefore, undertaken on fermentation in the filtrate mentioned and also in the filtrate from the mercury precipitation.

#### *Experiment No. 16.*

These experiments will not be given in detail; only the method of procedure and the results will be recorded. To the filtrate from the Zn-HCl precipitation, which was perfectly clear, primary phosphate was added until a sample removed showed a pH value between 5.5 and 7.0. Yeast and glucose were then added in the same relative amounts as usual. The urine was simultaneously fermented in the ordinary way. Several experiments gave the same result, namely, that fermentation proceeded much more slowly in the filtrate from the Zn-HCl precipitation than in

the original urine. As the experiments with the filtrate from the mercury precipitation exhibited a still more incomplete fermentation, the reason for the deficient fermentation in these filtrates probably was that they contained small quantities of mercury—more in that from the Hg precipitation than in that from the Zn—which is known to inhibit fermentation or bring it to a standstill, even in minimal amounts. The possibility of fermentation in the filtrate from the Zn precipitation instead of in the urine had, therefore, to be given up.

The fermentation in urine in the following experiments was, therefore, arranged in such a way that the pH of the urine was brought to between 5.5 and 6.8, if it was not already within this range. The urine was then brought to the boil, cooled, and about 1 pro mille glucose and about 5% fresh ordinary commercial yeast added. After remaining for 48 hours in the thermostat, the reducing bodies present were determined as previously described.

Fermentation in urine, even when the precautions mentioned above are taken, is far from being an ideal quantitative method, since besides being very clumsy it is rather inaccurate. Fermentation sometimes proceeds very slowly and is not infrequently disturbed by ammonia production. The results for fermented sugar in urine reported below must therefore be regarded as *minimal values*, which merely indicate the coarse fluctuations in the fermentable substances in the urine. But for the latter purpose the method is quite applicable.

In 1921, Benedict and Osterberg<sup>45</sup> published a modification of their method which consisted in treating the urine with "commercial bone black" in place of precipitating with mercury solution and Zn-HCl, and in adding a few drops of acetone as well as picric acid and alkali to the filtrate. In this way the color produced by the interaction of creatin and creatinin with picric acid is eliminated. Most of my experiments were concluded when the method was published, but I have nevertheless carried out some investigations with it.

#### *Experiment No. 17.*

0.5 pro mille watery glucose solution was shaken with pure animal charcoal (Merck), which had previously been treated with HCl as recommended by Benedict and Osterberg. After filtration it was found that 60% of the sugar was absorbed by the charcoal. The authors point out that too finely divided charcoal must not be used, and a fresh experiment was, therefore, made in which *carbo animalis dep. humidus* (Merck) was employed, which was likewise treated with boiling HCl as described by the authors. As this charcoal also retained the sugar, in considerably



less amount it is true (13%), and as no other kind could be got, further experiments with this method were abandoned.

This modification of Benedict and Osterberg's method also involves fermentation of the urine and a fresh determination in the fermented urine and as the fermentation, as will appear from the experiment reported above, is the most troublesome and the least accurate by Benedict and Osterberg's method, I am unable to appreciate that the method has gained very much by the modification, even providing that the authors had succeeded in introducing a kind of charcoal which does not absorb sugar.

The objection may be brought against the method published by Folin and Berglund<sup>25</sup> in 1922, referred to above, that we are not absolutely certain whether the reducing substances in the filtrate from the precipitation of the urine with "Lloyd's alkaloidal reagent" are sugars. Urine contains so many constituents, the nature of many of which is unknown, that a reduction reaction in the filtrate from a precipitation, even if the uric acid, creatinin, and creatin are removed in the process, is a rather uncertain measure of the normal sugar of the urine, especially because it is so small in amount.

#### *Hagedorn and Norman Jensen's Method.\**

This method for the quantitative estimation of blood sugar will not be discussed, and the reader is referred to the original article.<sup>38</sup> Suffice it to say, in the present experiments, it has given values which agree with one another quite well. Some drops of xylol are added to the thiosulphate solution so as to prevent the growth of organisms. Xylol does not affect the titration and the thiosulphate solution keeps constant if it is always present in sufficient quantity.

#### *The Qualitative Sugar Reactions.*

I will discuss briefly the qualitative reactions employed in the present work. *Benedict's reaction* was carried out by adding 5 cc. of Benedict's reagent to 0.5 cc. urine, after which it was heated in a water bath at 100°C for 5 minutes. If at the end of this time a thick green, yellow, or red precipitate appeared the reaction was called positive. If the liquid remained clear or only contained a slight precipitate the reaction was called negative.

*Almén's reaction* was done with 5 cc. urine and 0.5 cc. reagent, which was boiled over a gas flame for 2 minutes. If a dark brown, grayish black, or quite black precipitate formed the reaction was considered positive. The reaction was judged at least 5 minutes after boiling.

*The phenylhydrazin reaction* is described at length later (see "Investigations into the Occurrence of Glucose in Normal Urine").

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\* The method is translated into English in J. Biol. Chem., Vol. 42, p. 349.

In all the following experiments Benedict's reaction was used as the standard method for demonstrating sugar in urine and is denoted in the table by "Benedict."

In the following investigations are used Benedict and Osterberg's term, "total sugar": the amount of reducing bodies in the urine, estimated as described above; "non-fermentable sugar:" the fermentable bodies in the urine estimated as the difference between the total sugar and non-fermentable sugar. Whenever "urine sugar" or "amount of sugar" are mentioned, the fermentable sugar is always meant.

Blood sugar is always expressed in mg. per 100 cc. blood.

As a series of experimental persons is used several times, information about them is given here to avoid repetition.

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### PERSONS OF EXPERIMENT

No.

1. K. M., doctor, healthy, 27 years, 70 Kg.
2. F. J., doctor, healthy, 29 years, 66 Kg.
3. H. H., doctor, healthy, 37 years, 68 Kg.
4. A. B., doctor, healthy, 36 years, 90 Kg.
5. T. K., girl, diabetic, 9 years, 19 Kg.
6. M. G., woman, diabetic, 62 years, 65 Kg.
7. H. L., man, diabetic, 33 years, 65 Kg.
8. H. A., man, diabetic, 41 years, 65 Kg.
9. H. F., woman, diabetic, 56 years, 61 Kg.
11. J. E., man, convalescent, 22 years, 65 Kg.
12. E. K., man, convalescent, 24 years, 62 Kg.
15. K. L., medical student, healthy, 24 years, 70 Kg.
16. J., medical student, glycosuria, 24 years, 68 Kg.
17. L., medical student, healthy, 28 years, 72 Kg.
18. H. S., medical student, healthy, 30 years, 70 Kg.
19. G., medical student, healthy, 24 years, 70 Kg.
20. O., medical student, glycosuria, 27 years, 70 Kg.
21. O. M., medical student, healthy, 24 years, 73 Kg.
22. E. O., man, convalescent, 23 years, 56 Kg.
23. M., medical student, healthy, 25 years, 74 Kg.
24. E. L., man, convalescent, 21 years, 75 Kg.
25. N., medical student, healthy, 24 years, 73 Kg.
26. A. R., medical student, glycosuria, 27 years, 65 Kg.
27. R. R., man, convalescent, 28 years, 61 Kg.
29. H. H., man, convalescent, 27 years, 77 Kg.
30. E. H., doctor, healthy, 26 years, 63 Kg.
31. H. N., man, convalescent, 21 years, 71 Kg.
32. A. R., man, diabetic, 58 years, 59 Kg.
33. G. C., man, convalescent, 19 years, 60 Kg.
34. C., man, healthy, 40 years, 70 Kg.
35. J. H., man, convalescent, 62 years, 66 Kg.
36. B. S., man, convalescent, 20 years, 57 Kg.

37. K. R., man, convalescent, 38 years, 73 Kg.
38. K. M., man, convalescent, 23 years, 75 Kg.
39. O. L., man, convalescent, 28 years, 65 Kg.
40. H. F., man, convalescent, 33 years, 62 Kg.
41. O. P., man, convalescent, 46 years, 63 Kg.
42. H. H., man, convalescent, 20 years, 68 Kg.
43. H. O., man, diabetic, 22 years, 64 Kg.
44. H. T., doctor, glycosuria, 39 years, 72 Kg.
45. P. S., man, diabetic, 43 years, 68 Kg.
47. E. A., man, diabetic, 20 years, 52 Kg.
51. O. J., man, convalescent, 18 years.
58. L. B., man, vitium cordis, 67 years.
59. N. N., man, vitium cordis, 30 years, 70 Kg.
60. L. A., man, glycosuria, 31 years.
61. N. A., man, glycosuria, 30 years.
62. T., man, glycosuria, 40 years.
63. H. T., man, glycosuria, 39 years.
64. E. H., man, glycosuria, 34 years.

## II. *The Urine Sugar and Its Relation to the Blood Sugar in Healthy Persons.*

As healthy persons are reckoned convalescents and patients suffering from diseases which would be considered to have no effect on the sugar excretion, like paralyses, etc., as well as absolutely healthy individuals.

### *The Amount of Sugar in the 24-hour Urine.*

The urine was collected, which was passed from 7 in the morning to 7 the next morning. Where there was the slightest doubt about it, the sugar estimation was not undertaken.

#### *Experiment No. 18.*

##### Person of experiment No. 1.

Day	Urine cc.	Total sugar		Fermented	
		pro mil.	mg.	pro mil.	mg.
1	1310	0.61	779	0.26	341
2	1500	0.52	780	0.24	360
3	1480	0.63	932	0.40	592
4	1460	0.48	701	0.17	248
5	1540	0.40	666	0.15	231



*Experiment No. 19.*

## Person of experiment No. 2.

Day	Urine cc.	Total sugar		Fermented	
		pro mil.	mg.	pro mil.	mg.
1	740	1.30	962	0.50	370
2	830	0.80	664	0.40	320
3	1410	0.58	818	0.26	367
4	1350	0.76	1026	0.48	648
5	775	1.00	775	0.44	341
6	860	0.83	714	0.39	335
7	1000	0.90	900	0.40	400
8	1200	0.66	792	0.24	288

*Experiment No. 20.*

## Person of experiment No. 3.

Day	Urine cc.	Total sugar		Fermented	
		pro mil.	mg.	pro mil.	mg.
1	1630	0.49	799	0.17	277
2	775	1.06	822	0.39	302
3	780	1.10	855	0.32	250
4	1400	0.72	1008	0.34	476
5	1150	0.69	794	0.30	345
6	1040	0.89	926	0.31	322
7	1080	0.66	713	0.40	432

*Experiment No. 21.*

## Person of experiment No. 4.

Day	Urine cc.	Total sugar		Fermented	
		pro mil.	mg.	pro mil.	mg.
1	2350	0.40	940	0.14	329
2	1420	0.77	1.093	0.31	440
3	1200	0.86	1.032	0.33	396
4	1290	0.80	1.032	0.40	516
5	1700	0.58	986	0.20	340

*Experiment No. 22.*

## Person of experiment No. 12.

Day	Urine cc.	Total sugar		Fermented	
		pro mil.	mg.	pro mil.	mg.
1	1000	0.45	450	0.18	180
2	1000	0.50	500	0.20	200
3	1275	0.47	599	0.21	268
4	1550	0.37	573	0.12	186
5	1500	0.43	645	0.17	255
6	1550	0.56	868	0.20	310
7	1250	0.81	1.125	0.21	263

*Experiment No. 23.*

## Person of experiment No. 16.

Day	Urine cc.	Total sugar		Fermented	
		pro mil.	mg.	pro mil.	mg.
1	1530	0.75	1.148	0.30	459
2	900	1.33	1.197	0.53	477
3	880	0.50	1.320	0.55	484

*Experiment No. 24.*

## Person of experiment No. 17.

Day	Urine cc.	Total sugar		Fermented	
		pro mil.	mg.	pro mil.	mg.
1	1480	0.88	1.302	0.48	710
2	1680	0.70	1.176	0.32	538

As will be seen, 7 men were examined for 37 days, during which time they lived on ordinary diet. Their ages varied between 23 and 37 years, and their weights between 62 and 90 kg. The total sugar excretion varied between 450 and 1,320 mg., the mean being 871 mg., while the excretion of fermentable sugar varied between 180 and 710 mg., the mean being 368 mg. The excretion varied considerably, not only from one person to another, but also in the same individual. There seems to be some relation to body-weight because Nos. 21 and 24, who weighed 90 and 75 kg., respectively, showed a larger excretion than the others with the exception of No. 16.

The fermentable sugar constituted rather less than half the total sugar.

The investigations into *the relation of the urine sugar to various factors*, such as the absorption of water, meals, etc., were usually carried out in the morning before breakfast, that is, 12-14 hours after the last meal. Urine was collected an hour before the experiment and used for the comparison, and during the experiment urine was collected every 15 minutes usually for 2-3 hours after the experiment was started.

Before these experiments were begun an investigation was made as to whether the sugar excretion in a person fasting in the morning was fairly constant from hour to hour.

*Experiment No. 25.*

## Person of experiment No. 37.

				Urine	Total sugar		Fermented	
				cc.	pro mil.	mg.	pro mil.	mg.
The average output per								
hour from 9 p. m. to 6 a. m.						37		19
6 a. m. to	7 a. m.			55	0.45	25	0.18	10
7 “ “	8 “			70	0.30	21	0.12	8
8 “ “	9 “			70	0.30	21	0.11	8

*Experiment No. 26.*

Person of experiment No. 39.

	Urine cc.	Total sugar pro mil.	mg.	Fermented pro mil.	mg.
The average output per hour from 9 p. m. to 6 a. m.			30		20
6 a. m. to 7 a. m.	60	0.40	24	0.20	12
7 " " 8 "	55	0.40	22	0.18	10
8 " " 9 "	46	0.44	20	0.24	11

*Experiment No. 27.*

Person of experiment No. 35.

	Urine cc.	Total sugar pro mil.	mg.	Fermented pro mil.	mg.
6 a. m. to 7 a. m.	60	0.40	24	0.18	11
7 " " 8 "	30	0.78	23	0.40	12
8 " " 9 "	31	0.75	23	0.40	12
9 " " 10 "	28	0.84	24	0.54	15

It is evident from the 3 experiments that the sugar output in the individual is rather constant from hour to hour, but in different individuals the output varied much. In two of the experiments the average sugar output per hour during the night was determined. In both experiments the output was greater in the night hours than in the morning hours.

*Investigations on the Influence of Water Ingestion.**Experiment No. 28.*

Person of experiment No. 33.

	Urine cc.	Total sugar pro mil.	mg.	Fermented pro mil.	mg.
7 a. m., 300 cc. of water					
6 a. m. to 7 a. m.	50	0.70	35	0.25	13
7 " " 8 "	175	0.30	53	0.12	21
8 " " 9 "	80	0.49	39	0.21	10
9 " " 10 "	86	0.43	37	0.14	12

*Experiment No. 29.*

Person of experiment No. 35.

	Urine cc.	Total sugar pro mil.	mg.	Fermented pro mil.	mg.
7 a. m., 300 cc. of water					
6 a. m. to 7 a. m.	65	0.50	33	0.20	13
7 " " 8 "	105	0.40	42	0.18	19
8 " " 9 "	50	0.57	29	0.24	12
9 " " 10 "	31	0.86	26	0.31	10



*Experiment No. 30.*

Person of experiment No. 40.

	Urine cc.	Total sugar		Fermented	
		pro mil.	mg.	pro mil.	mg.
8 a. m., 200 cc. of water					
7 a. m. to 8 a. m.	41	0.44	18	0.20	8
8 " " 9 "	62	0.33	20	0.13	8
9 " " 10 "	88	0.21	18	0.10	9
10 " " 11 "	60	0.29	17	0.12	7

*Experiment No. 31.*

Person of experiment No. 36.

	Urine cc.	Total sugar		Fermented	
		pro mil.	mg.	pro mil.	mg.
8 a. m., 200 cc. of water					
7 a. m. to 8 a. m.	30	1.0	30	0.30	9
8 " " 9 "	40	0.75	30	0.23	9
9 " " 10 "	46	0.67	31	0.15	7
10 " " 11 "	40	0.63	25	0.20	8

*Experiment No. 32.*

Person of experiment No. 41.

	Urine cc.	Total sugar		Fermented	
		pro mil.	mg.	pro mil.	mg.
8 a. m., 200 cc. of water					
7 a. m. to 8 a. m.	36	0.48	17	0.20	7
8 " " 9 "	88	0.23	20	0.10	9
9 " " 10 "	123	0.13	16	0.06	7
10 " " 11 "	82	0.25	17	0.11	9

In the first two experiments the increased urine output was accompanied by an increase of both the total sugar and the fermentable sugar, but in the last three experiments there was no increase of the sugar output, although the diuresis was augmented in the last experiment. An increase of the sugar output, which accompanies a pronounced increase of the water elimination may, therefore, be produced by the increased diuresis.

*Investigations on the Influence of Acids.**Experiment No. 33.*

Person of experiment No. 11.

9 a. m. : 2 cc. of dilute HCl  
mixed with 300 cc. of water

	Urine cc.	Total sugar		Fermented		Blood sugar
		pro mil.	mg.	pro mil.	mg.	
7 a. m. to 9 a. m.	210	0.25	53	0.12	26	9 a. m. : 83
9 " " 11 "	310	0.18	56	0.07	22	9.30 " : 80
						10 " : 83

*Experiment No. 34.*

Person of experiment No. 33.

8 a. m. : 2 cc. of dilute HCl  
mixed with 300 cc. of water

		Urine	Total sugar		Fermented		Blood sugar
		cc.	pro mil.	mg.	pro mil.	mg.	
7 a. m. to	8 a. m.	65	0.47	31	0.20	13	8.30 a. m. : 89
8 "	" 9 "	213	0.22	47	0.08	17	9 " : 98
9 "	" 10 "	125	0.23	35	0.10	13	9.30 " : 94
10 "	" 11 "	82	0.35	29	0.12	10	10 " : 85
							10.30 " : 84
							11 " : 86

*Experiment No. 35.*

Person of experiment No. 56.

8 a. m. : 2 cc. of dilute HCl  
mixed with 200 cc. of water

		Urine	Total sugar		Blood sugar
		cc.	pro mil.	mg.	
7 a. m. to	8 a. m.	30	0.41	12	8 a. m. : 89
					8.15 " : 89
8 "	" 9 "	58	0.26	15	8.30 " : 89
					8.45 " : 89
9 "	" 10 "	45	0.34	15	9 " : 88
					9.15 " : 89
					9.30 " : 89
					9.45 " : 88
					10 " : 89

In experiment No. 34, there was a considerable increase in diuresis and simultaneously an increase in the total sugar and the fermentable sugar, but as both the latter may be due to the increased diuresis, as is evident from the preceding experiment, the hydrochloric acid cannot be made responsible for it. In the other two cases, there was no change in the sugar excretion. The blood sugar was estimated in all the three cases. In experiment No. 34, there was a rise in the blood sugar at about the same time as the increased sugar excretion took place, but it was not great and in neither of the two other cases was there any demonstrable change in the blood sugar concentration.

*Investigations on the Influence of Alkalies.*

The influence of alkalies on the physiologic sugar elimination was tested by giving 20 grams of soda bicarbonate. The sugar output was determined partly in the 24 hour urine, partly in the urine per hour in the morning.

*Experiment No. 36.*

Person of experiment No. 57.

The 4th day 20 gm. of sodium bicarbonate were given.

Day	Urine	Total sugar		Fermented	
	cc.	pro mil.	mg.	pro mil.	mg.
1	1250	0.70	875	0.35	437
2	1750	0.33	575	0.15	262
3	1600	0.53	848	0.26	416
4	1500	0.45	675	0.20	300
5	1400	0.50	700	0.22	308

*Experiment No. 37.*

Person of experiment No. 12.

The 6th day 20 gm. of sodium bicarbonate were given.

Day	Urine	Total sugar		Fermented	
	cc.	pro mil.	mg.	pro mil.	mg.
1	1275	0.47	599	0.21	268
2	1550	0.37	573	0.12	186
3	1500	0.43	645	0.17	255
4	1550	0.56	868	0.20	310
5	1250	0.81	1125	0.21	263
6	1400	0.60	840	0.30	420
7	1300	0.65	845	0.30	339

*Experiment No. 38.*

Person of experiment No. 12.

9/24 the urine was collected for comparison.

	Urine	Total sugar		Fermented	
	cc.	pro mil.	mg.	pro mil.	mg.
September 24th					
6 a. m. to 8 a. m.	104	0.57	59	0.18	19
8 " " 10 "	110	0.47	52	0.22	24

9/25, 8 a. m., 20 gm. of sodium bicarbonate were given mixed with 200cc. of water.

	Urine	Total sugar		Fermented	
	cc.	pro mil.	mg.	pro mil.	mg.
September 25th					
6 a. m. to 8 a. m.	65	1.06	69	0.27	18
8 " " 10 "	120	0.45	54	0.15	18



*Experiment No. 39.*

Person of experiment No. 2.

The 4th day 20 gm. of sod. bicarb.

Day	Urine cc.	Total sugar		Fermented	
		pro mil.	mg.	pro mil.	mg.
1	1550	0.63	977	0.33	512
2	920	0.75	690	0.37	340
3	1500	0.54	810	0.24	360
4	1160	1.73	2007	1.14	1322
5	1030	1.30	1339	0.60	618

*Experiment No. 40.*

Person of experiment No. 4.

The 3rd day 20 gm. of sod. bicarb.

Day	Urine cc.	Total sugar		Fermented	
		pro mil.	mg.	pro mil.	mg.
1	1500	0.60	900	0.25	375
2	1750	0.55	963	0.20	350
3	2100	0.77	1617	0.50	1050
4	1600	0.50	800	0.20	320

*Experiment No. 41.*

Person of experiment No. 33.

9 a. m., 20 gm. of sod. bicarb. with 300 cc. of water.

February 27th	Urine cc.	Total sugar		Blood sugar	
		pro mil.	mg.		
8 a. m. to 9 a. m.	32	0.98	31	9 a. m. :	88
9 " " 10 "	35	1.0	35	9.30 " :	88
				10 " :	88
10 " " 11 "	82	0.58	48	10.30 " :	90
				11 " :	89
11 " " 12 "	70	0.62	43	11.30 " :	88
				12 " :	89

The 6 experiments relating to the administration of sodium bicarbonate, as will be seen, have not given uniform results. In experiments 39 and 40, there was a considerable increase of the total sugar and the fermentable sugar on the day the sodium bicarbonate was given, while experiments 36 and 37 did not show any alteration in the excretion. In experiment 41, the total sugar rose somewhat, but the diuresis at the same time increased to twice its amount, which would explain the increase in the sugar excretion as already indicated. The blood sugar was estimated only in experiment 41 and showed no change after the administration of alkali.

*Investigations on the Influence of Mixed Meals.**Experiment No. 42.*

Person of experiment No. 3.

11.30 a. m., breakfast: Bread, butter, cheese, coffee.

2.30 p. m., dinner: Meat, potatoes, beans, fruit, milk.

	Urine		Total sugar		Blood sugar
	cc.	Benedict	pro mil.	mg.	
10.30 to 11.30 a. m.	47	—	0.50	24	
11.30 " 12.30 "	48	—	0.90	43	12 noon : 126
12.30 " 1.30 p. m.	60	—	0.70	42	1 p. m. : 121
1.30 " 2.30 "	65	—	0.60	39	2 " : 100
2.30 " 3.30 "	88	—	0.85	75	3 " : 147
3.30 " 4.30 "	100	—	0.68	68	4 " : 117

*Experiment No. 43.*

Person of experiment No. 12.

9 a. m., breakfast: Milk, bread, butter, eggs.

	Urine		Total sugar		Fermented	Blood sugar
	cc.	pro mil.	mg.	pro mil.	mg.	
7 a. m. to 9 a. m.	55	0.66	36	0.25	14	9 a. m. : 90
						9.30 " : 114
9 " " 11 "	85	0.83	71	0.58	62	10 " : 107
						10.30 " : 99
						11 " : 100

*Experiment No. 44.*

Person of experiment No. 18.

10 a. m., breakfast.

2 p. m., dinner: Sirloin, cabbage, potatoes, orange.

	Urine		Total sugar		Blood sugar
	cc.	pro mil.	mg.		
1.30 to 2 p. m.	21	0.43	9		1.45 p. m. : 101
2 " 2.30 "	26	0.47	12		2.15 " : 113
2.30 " 3 "	40	0.70	28		2.45 " : 128
3 " 3.30 "	43	0.70	30		3.15 " : 91
3.30 " 4 "	40	0.65	26		3.45 " : 84

In the three experiments on the effect of mixed diets on the urine sugar, the total sugar rose considerably after each meal—both breakfast and the midday meal. In one of the experiments the fermentable sugar was estimated and showed a very considerable increase. The blood sugar exhibited a rise after every meal, but there was no distinct parallelism with the urine sugar. In experiment 44, the blood sugar attained its maximum before the urine sugar.

*Investigations into the Influence of Single Articles of Food.*

The foodstuffs that were particularly investigated were different kinds of bread, as wheat bread (French bread), ordinary fine sifted rye bread, ground rye bread ("coarse bread"), and lastly brown bread (bran bread). The effect of meat, green vegetables and soup was also investigated.

*Experiment No. 45.*

Person of experiment No. 22.

8 a. m.: 100 gm. of ordinary rye bread and 200 cc. of water.

	Urine cc.	Bene- dict	Total sugar pro mil.	mg.	Fermented pro mil.	mg.	blood sugar
7 to 8 a. m.	38	—	0.47	18	0.20	8	8 a. m. : 80
							8.30 " : 121
8 " 9 "	34	—	0.90	31	0.40	14	9 " : 133
							9.30 " : 126
9 " 10 "	28	+	1.29	36	0.60	17	10 " : 119
							10.30 " : 118

*Experiment No. 46.*

Person of experiment No. 28.

8 a. m.: 100 gm. of ordinary rye bread and 200 cc. of water.

	Urine cc.	Benedict	Total sugar pro mil.	mg.	Blood sugar
7 a. m. to 8 a. m.	30	—	0.50	15	8 a. m. : 101
8.15 a. m.	7	—	0.60	16	101
8.30 "	6	—			112
8.45 "	6.5	—			129
9 "	6.5	—			126
9.15 "	5	—	0.75	17	126
9.30 "	6	—			125
9.45 "	6	—			125
10 "	6	—			119

*Experiment No. 47.*

Person of experiment No. 28.

8 a. m.: 100 gm. of bran bread and 200 cc. of water.

	Urine cc.	Benedict	Total sugar pro mil.	mg.	Blood sugar
7 a. m. to 8 a. m.	34	—	0.35	12	8 a. m. : 90
8.15 a. m.	10	—	0.45	16	101
8.30 "	10	—			101
8.45 "	6	—			105
9 "	9	—			105
9.15 "	13	—	0.39	21	105
9.30 "	17	—			99
9.45 "	11	—			86
10 "	14	—			85



*Experiment No. 48.*

Person of experiment No. 29.

8 a. m.: 100 gm. of bran bread and 200 cc. of water.

	Urine cc.	Benedict	Total sugar		Blood sugar
			pro mil.	mg.	
7 a. m. to 8 a. m.	34	—	0.39	13	8 a. m. : 85
8.15 a. m.	12	—	0.50	22	95
8.30 "	10	—			105
8.45 "	7	—			100
9 "	5	—			100
9.15 "	8.5	—	0.50	21	95
9.30 "	10	—			95
9.45 "	10	—			96
10 "	13	—			96

*Experiment No. 49.*

Person of experiment No. 31.

8 a. m.: 100 gm. of bran bread and 200 cc. of water.

	Urine cc.	Bene- dict	Total sugar		Fermented		Blood sugar
			pro mil.	mg.	pro mil.	mg.	
7 a. m. to 8 a. m.	56	—	0.34	19	0.14	8	94
8.15 a. m.	18	—	0.58	34	0.23	13	96
8.30 "	15	—					99
8.45 "	15	—					115
9 "	10	—					110
9.15 "	10	—	1.25	50	0.50	20	105
9.30 "	10	—					103
9.45 "	10	—					95
10 "	10	—					91
10 to 11 a. m.	27	+	1.64	44	0.85	23	10.15 : 88
							10.30 : 90
							10.45 : 92
							11 : 91

*Experiment No. 50.*

Person of experiment No. 33.

8 a. m.: 100 gm. of bran bread and 200 cc. of water.

	Urine cc.	Bene- dict	Total sugar		Fermented		Blood sugar
			pro mil.	mg.	pro mil.	mg.	
7 a. m. to 8 a. m.	23	—	1.12	26	0.52	12	8 : 89
8.30 a. m.	15	—	1.78	45	0.75	18	8.30 : 97
9 "	10	+					9 : 92
9.30 "	13	+	2.65	61	1.0	23	9.30 : 88
10 "	10	+					10 : 89
10.30 "	12	+	3.15	72	1.5	35	10.30 : 89
11 "	11	+					11 : 88

Experiment No. 51.

Person of experiment No. 33.

8 a. m.: 100 gm. of wheat bread and 200 cc. of water.

	Urine cc.	Bene- dict	Total sugar pro mil.	mg.	Fermented pro mil.	mg.	Blood sugar
7 a. m. to 8 a. m.	64	—	0.50	32	0.19	12	8 : 88
8.30 a. m.	29	—	0.74	41	0.34	19	8.30 : 115
9 "	27	—					9 : 94
9.30 "	74	—	0.42	61	0.17	24	9.30 : 88
10 "	70	—	0.40	55	0.12	17	10 : 85
10.30 "	78	—					10.30 : 86
11 "	60	—					11 : 86

Experiment No. 52.

Person of experiment No. 33.

8 a. m.: 100 gm. of bran bread and 1,000 cc. of water.

10 a. m.: 300 cc. of water.

	Urine cc.	Bene- dict	Total sugar pro mil.	mg.	Fermented pro mil.	mg.	Blood sugar
7 a. m. to 8 a. m.	95	—	0.58	55	0.25	24	
8.30 a. m.	33	—	0.20	62	0.10	31	8.30 : 95
9 "	275	—					9 : 92
9.30 "	275	—	0.27	85	0.12	38	9.30 : 88
10 "	40	—	0.28	120	0.14	60	10 : 87
10.30 "	200	—					10.30 : 87
11 "	230	—					11 : 89

Experiment No. 53.

Person of experiment No. 34.

9 a. m.: 100 gm. of bran bread and 200 cc. of water.

	Urine cc.	Benedict	Total sugar pro mil.	mg.	Blood sugar
8 a. m. to 9 a. m.	24	—	0.70	17	9 a. m. : 96
9.30 a. m.	12	—	1.0	29	9.30 " : 116
10 "	17	—			10 " : 95
10.30 "	18	—	1.0	35	10.30 " : 93
11 "	17	—	1.18	33	11 " : 94
11.30 "	15	—			11.30 " : 93
12 "	15	+			

*Experiment No. 54.*

Person of experiment No. 35.

8 a. m.: 100 gm. of bran bread and 200 cc. of water.

		Urine cc.	Bene- dict	Total sugar pro mil.	sugar mg.	Fermented pro mil.	mg.	Blood sugar
7 a. m. to	8 a. m.	40	—	0.82	33	0.40	16	8 a. m. : 89
8 "	" 9 "	40	—	1.0	40	0.45	18	9 " : 112
9 "	" 10 "	20	+	2.5	50	1.70	34	10 " : 102
10 "	" 11 "	20	+	2.5	50	1.80	36	11 " : 82

*Experiment No. 55.*

Person of experiment No. 35.

8 a. m.: 100 gm. of wheat bread and 200 cc. of water.

		Urine cc.	Bene- dict	Total sugar pro mil.	sugar mg.	Fermented pro mil.	mg.	Blood sugar
7 a. m. to	8 a. m.	23	—	1.18	27	0.46	11	
8 "	" 9 "	32	—	1.00	32	0.30	10	9 a. m. : 130
9 "	" 10 "	25	—	1.30	33	0.50	13	10 " : 109
10 "	" 11 "	29	—	1.25	36	0.50	15	11 " : 95

*Experiment No. 56.*

Person of experiment No. 35.

8 a. m.: The ash of 100 gm. of bran bread and 200 cc. of water.

		Urine cc.	Bene- dict	Total sugar pro mil.	sugar mg.	Fermented pro mil.	mg.	Blood sugar
7 a. m. to	8 a. m.	25	—	1.00	25	0.50	13	
8 "	" 9 "	37	—	0.75	28	0.40	15	9 a. m. : 92
9 "	" 10 "	35	—	0.75	26	0.26	13	
10 "	" 11 "	49	—	0.53	26	0.26	13	

*Experiment No. 57.*

Person of experiment No. 36.

8 a. m.: 100 gm. of bran bread and 200 cc. of water.

		Urine cc.	Bene- dict	Total sugar pro mil.	sugar mg.	Fermented pro mil.	mg.	Blood sugar
7 a. m. to	8 a. m.	40	—	0.90	36	0.40	16	
8 "	" 9 "	50	—	0.90	45	0.40	20	100
9 "	" 10 "	38	+	1.62	62	0.90	34	78
10 "	" 11 "	37	+	1.90	62	1.00	37	80



Experiment No. 58.

Person of experiment No. 36.

8 a. m.: 100 gm. of wheat bread and 200 cc. of water.

				Urine	Bene-	Total sugar		Fermented		Blood sugar		
				cc.	dict	pro	mg.	pro	mg.			
						mil.		mil.				
7	a. m. to	8	a. m.	34	—	0.80	27	0.20	7	8	a. m. :	93
8	" "	8.30	"	15	—	0.57	34	0.12	7			
8.30	" "	9	"	44	—					9	" :	116
9	" "	9.30	"	23	—	0.63	42	0.18	12	9.30	" :	133
9.30	" "	10	"	44	—					10	" :	100
10	" "	10.30	"	62	—	0.45	46	0.13	13	10.30	" :	84
10.30	" "	11	"	41	—					11	" :	84

Experiment No. 59.

Person of experiment No. 37.

8 a. m.: 100 gm. of bran bread and 200 cc. of water.

				Urine	Bene-	Total sugar		Fermented		Blood sugar		
				cc.	dict	pro	mg.	pro	mg.			
						mil.		mil.				
7	a. m. to	8	a. m.	57	—	0.38	22	0.15	9			
8	" "	9	"	54	—	0.95	51	0.30	16	9 a. m. :		104
9	" "	10	"	92	—	0.75	69	0.31	29	10 "	:	97
10	" "	11	"	76	—	0.90	68	0.36	27	11 "	:	95

Experiment No. 60.

Person of experiment No. 37.

8 a. m.: 100 gm. of ground rye bread and 200 cc. of water.

				Urine	Bene-	Total sugar		Fermented		Blood sugar		
				cc.	dict	pro	mg.	pro	mg.			
						mil.		mil.				
7	a. m. to	8	a. m.	48	—	0.52	25	0.20	10			
8	" "	9	"	35	—	0.75	26	0.30	11	9.30 a. m. :		107
9	" "	10	"	41	—	0.85	35	0.35	14	9.30 "	:	88
										10	" :	88
10	" "	11	"	42	—	0.85	37	0.34	14	10.30 "	:	89
										11	" :	88

In the night before the experiment the average output per hour of total sugar was 32 mg. and that of fermented sugar 13 mg.

*Experiment No. 61.*

Person of experiment No. 37.

8 a. m.: 250 gm. of ground rye bread and 200 cc. of water.

		Urine	Bene-	Total sugar	Fermented		Blood sugar	
		cc.	dict	pro mil.	mg.	pro mil.	mg.	
7 a. m. to	8 a. m.	24	—	1.24	29	0.45	11	
8 " "	9 " "	23	—	1.34	31	0.50	12	9 a. m. : 96
								9.30 " : 95
9 " "	10 " "	20	trace	1.80	36	0.80	16	10 " : 90
								10.30 " : 90
10 " "	11 " "	21	trace	2.30	48	1.60	34	11 " : 91
11 " "	12 " "	18	trace	2.10	38	1.00	18	12 " : 80

In the night before the experiment the average output per hour of total sugar was 30 mg.

*Experiment No. 62.*

Person of experiment No. 37.

8 a. m.: 100 gm. of bran bread and 200 cc. of water.

		Urine		Total sugar		Fermented	
		cc.	Benedict	pro mil.	mg.	pro mil.	mg.
7 a. m. to	8 a. m.	48	—	0.72	35	0.31	15
8 “ “	9 “	35	—	1.21	42	0.49	17
9 “ “	10 “	38	+	1.55	59	0.71	27
10 “ “	11 “	29	+	2.25	65	1.0	29
11 “ “	12 “	19	+	2.68	51	1.20	23

In the night before the experiment the average output per hour of total sugar was 49 mg. and that of fermented sugar 19 mg.

*Experiment No. 63.*

Person of experiment No. 37.

8 a. m.: 210 gm. of wheat bread and 200 cc. of water.

		Urine	Bene-	Total sugar	Fermented		Blood sugar	
		cc.	dict	pro mil.	mg.	pro mil.	mg.	
7 a. m. to	8 a. m.	33	—	0.90	30	0.50	17	9 a. m. : 88
								9.30 " : 89
8 " "	9 " "	52	—	0.60	31	0.35	18	10 " : 90
								10.30 " : 88
9 " "	10 " "	52	—	0.75	39	0.41	21	11 " : 89
10 " "	11 " "	45	—	0.80	36	0.40	18	

In the night before the experiment the average output per hour of total sugar was 38 mg. and that of fermented sugar 22 mg.





Of the four sorts of bread, wheat bread has been used in 4 experiments—Nos. 33, 55, 58 and 63. Three times was given 100 gm. and once 210 gm. together with 200 cc. of water. In all four experiments there was an increase of total sugar and of fermentable sugar. The increase, however, was slight in experiments 55 and 63.

In the two first experiments the excretion was greatest during the 2nd hour after the beginning of the experiment, but in the other two experiments it was greatest during the 3rd hour. The largest excretion per hour of fermentable sugar was 24 mg. Benedict's qualitative sugar reaction was negative in all the urine samples in these experiments. The blood sugar rise after administration of white bread will be seen from the table below.

Experiment		Greatest blood sugar concentration after beginning of experiment	The blood sugar was again less than 110 mg. in:
No.	mg.		
51	115	30 min.	60 min.
55	130	60 "	2 hours
58	133	1½ hour	2 "
63	no increase of the blood sugar		

In experiment 63, which was not associated with any blood sugar rise, 210 gm. white bread was given, i. e., more than twice as much as in the other three experiments.

*Finely sifted rye bread* was used in 2 experiments, 45 and 46. In experiment 45 there was a distinct increase in the blood sugar and in the fermentable sugar of the urine. In experiment 46, there was no increase in the total sugar and so the fermentable sugar was not determined. In experiment 45, Benedict's qualitative sugar reaction was positive with a total sugar concentration of 1.29 pro mille and a concentration of fermentable sugar of 0.60 pro mille.

The blood sugar rise will be seen from the table.

Experiment		Greatest blood sugar concentration after beginning of experiment	
No.	mg.		
45	133	1 hour	In none of these experiments was the blood sugar less than 110 mg. in two hours.
46	129	45 min.	

*Coarse bread* was given in two experiments, 60 and 61. In one case 100 gm. was given and in the other 250 gm., combined in each case with 200 cc. of water. In both experiments the sugar excretion was increased, and was greatest after 250 gm. bread. In experiment 60, it was about the same during the 2nd and 3rd hours. In experiment 61, it was greatest during the 3rd hour and decreased again in the 4th hour. In experiment 60, the blood sugar rose to 107, but there was no definite increase in the second experiment.

*Brown bread* was used in 13 experiments, always in a quantity of 100 gm., and in addition 200 cc. water was drunk, with the exception of experiment 52, in which 1,300 cc. water was taken. All these 13 experiments with brown bread were associated with an increased excretion of total sugar and fermentable sugar. The excretion was usually considerably greater than in the above mentioned experiments with the other kinds of bread. The concentration of fermentable sugar in the hour's urine was greater than 1.5 pro mille on 4 occasions, and on 7 the excretion was greater than 30 mg. per hour. In 3 experiments the excretion was estimated only during 2 hours after the beginning of the experiment. In two of these the excretion was about equally great in the first and second hours; in the third, the excretion was greatest during the second hour. In the 10 remaining experiments the excretion was determined during 3 or 4 hours after the start of the experiment. In 8 experiments it was greatest during the 3rd hour, while in 2 it was about the same during the 2nd and 3rd hours.

Benedict's qualitative sugar reaction was positive no fewer than 16 times in the urine after the brown bread. The lowest concentrations of total blood sugar and fermentable sugar, with which Benedict's sugar reaction was positive, were 1.40 and 0.60 pro mille, respectively.

Prior to 5 of the experiments, the average sugar excretion per hour in the night was estimated. This proved to be rather greater in all cases than during fasting in the morning.

In one experiment, No. 56, instead of 100 gm. brown bread, the same amount incinerated was given. As will be seen, it had no effect on the sugar excretion.

The behavior of the blood sugar in the experiments with brown bread will be seen from the following table:

Experiment		Greatest blood sugar concentration after beginning of experiment	The blood sugar was again less than 110 mg. in
No.	mg.		
47	105	45 to 75 min.	
48	105	30 min.	
49	115	45 "	75 min.
50	97	30 "	
52	no increase		
53	116	30 "	60 "
54	112	60 "	2 hours
57	100	60 "	
59	104	60 "	
64	no increase		
65	103	60 "	
66	100	30 "	

It is evident that the blood sugar rise was not great in any experiment, and only in three experiments was the sugar concentration more than 110 mg. per 100 cc. of blood.

The greatest blood sugar rise was always in the first hour, while the greatest sugar output, as a rule, was in the third hour.

*Experiment No. 67.*

Person of experiment No. 33.

8 a. m.: 390 gm. meat, 100 gm. cabbage and 200 cc. water.

	Urine cc.	Benedict	Total sugar pro mil.	mg.	Blood sugar
7 a. m. to 8 a. m.	87	—	0.44	38	
					8.30 a. m. : 87
8 " " 9 "	62	—	0.56	35	9 " : 89
					9.30 " : 90
9 " " 10 "	95	—	0.45	43	10 " : 90
					10.30 " : 90
10 " " 11 "	60	—	0.57	34	11 " : 92

*Experiment No. 68.*

Person of experiment No. 37.

8 a. m.: 390 gm. meat, 150 gm. cabbage and 200 cc. water.

	Urine	Bene- dict	Total sugar pro mil.	mg.	Fermented pro mil.	mg.	Blood sugar
7 a. m. to 8 a. m.	35	—	0.72	25	0.30	11	9 a. m. : 89
8 " " 9 "	53	—	0.60	31	0.20	11	10 " : 87
9 " " 10 "	42	—	0.62	26	0.25	11	11 " : 88
10 " " 11 "	40	—	0.80	32	0.35	14	12 " : 87
11 " " 12 "	28	—	1.10	31	0.40	11	

In the night before the experiment the average output per hour of total sugar was 31 mg. and that of fermented sugar 15 mg.

*Experiment No. 69.*

Person of experiment No. 40.

8 a. m.: 300 gm. meat and 200 cc. water.

	Urine cc.	Benedict	Total sugar pro mil.	mg.	Fermented pro mil.	mg.
7 a. m. to 8 a. m.	38	—	0.50	19	0.24	9
8 " " 9 "	24	—	0.76	18	0.33	8
9 " " 10 "	52	—	0.50	26	0.28	10
10 " " 11 "	67	—	0.40	27	0.20	13

*Experiment No. 70.*

Person of experiment No. 40.

8 a. m.: 300 gm. meat and 200 cc. water.

	Urine cc.	Benedict	Total sugar pro mil.	mg.	Fermented pro mil.	mg.
7 a. m. to 8 a. m.	52	—	0.27	14	0.11	6
8 " " 9 "	48	—	0.36	17	0.17	8
9 " " 10 "	54	—	0.35	19	0.17	9
10 " " 11 "	50	—	0.44	22	0.20	10



*Experiment No. 71.*

Person of experiment No. 51.

8 a. m.: 300 gm. meat, 100 gm. cabbage and 200 cc. water.

		Urine		Total sugar		
		cc.	Benedict	pro mil.	mg.	Blood sugar
7 a. m. to	8 a. m.	72	—	0.28	20	8 a. m. : 92
8 “ “	9 “	99	—	0.20	20	8.30 “ : 93
9 “ “	10 “	73	—	0.33	24	9 “ : 95
						9.30 “ : 94
10 “ “	11 “	50	—	0.45	23	10 “ : 93
						10.30 “ : 94
						11 “ : 92

*Experiment No. 72.*

Person of experiment No. 51.

9 a. m.: 150 gm. cabbage and 300 cc. broth.

		Urine	Total sugar		Fermented		
		cc.	pro mil.	mg.	pro mil.	mg.	Blood sugar
8 a. m. to	9 a. m.	52	0.32	17	0.10	5	9 a. m. : 93
							9.15 “ : 93
9 “ “	10 “	48	0.34	16	0.12	6	9.30 “ : 94
							9.45 “ : 93
10 “ “	11 “	70	0.32	22	0.10	7	10 “ : 95
							10.15 “ : 94
							10.30 “ : 94
							10.45 “ : 95
							11 “ : 94

Of six experiments with meat, cabbage and water or soup, two, Nos. 69 and 70, were arranged to include meat and water only, while three, Nos. 67, 68 and 70, included meat, cabbage and water, and the last, No. 72, included only cabbage and soup.

Experiments 69 and 70 were carried out on the same person and under exactly the same conditions. They both show a distinct, but not great, increase in the sugar excretion.

The experiments with meat, cabbage and water, and the experiment with cabbage and water, on the other hand, show no alteration in the sugar excretion.

The blood sugar was quite unaffected by these test meals.

*The Effect of the Administration of Glucose by Mouth on the Urine Sugar and Blood Sugar.*

It is clear from the preceding experiments that the *physiological sugar excretion* is partly independent of the blood sugar concentration, for it was shown that the sugar excretion could vary

within rather wide limits without any detectable change occurring in the blood sugar, and the increase of the sugar in the blood and urine took place at different times, the maximal blood sugar concentration occurring, almost without exception, during the first hour of the experiment while the maximal sugar excretion usually happened in the 3rd hour. These experiments, however, were not suitable, for several reasons, for an accurate study of the relation between blood sugar and urine sugar. Firstly, it might be imagined that the increased sugar excretion, which took place particularly after bread meals, was not due to glucose but to other kinds of sugar, as Folin and Berglund<sup>24</sup> think, and that the different methods used in the blood and urine investigations made a comparison between the values found for blood sugar and urine sugar impossible. Secondly, it might be thought, as Benedict and Osterberg<sup>18, 19</sup> hold, that digestion, as such, could influence the physiological sugar excretion. Lastly, the variations in the blood sugar which occurred after bread meals were relatively small. All these facts, in association with the desire to carry out some experiments on the relation of clinical glycosuria to the blood sugar, and on the relation between physiological sugar excretion and clinical glycosuria, made a series of experiments on the administration of glucose necessary. In such experiments the effect of digestion can be put out of court and the difference in technique will at the same time lose its importance, since glucose can be recovered quantitatively in the urine by Benedict and Osterberg's method, as my experiments prove.

In tolerance experiments glucose may be introduced into the organism in two ways—by the mouth or intravenously. A third method—per rectum—is inapplicable because absorption from the lowest part of the intestine is much too variable. In the case of oral administration the objection may justly be raised that absorption may take place with variable rapidity, which may affect the blood sugar rise and through it the appearance of glycosuria. Woodyatt, Sansum, and Wilder<sup>46, 47</sup> have therefore introduced definite amounts of glucose intravenously in unit intervals of time with an automatic pump, and they found that glycosuria occurs in normal persons when they receive between 0.8 and 0.9 gm. glucose per kg. body-weight per hour. This method is, however, much too complicated for general applica-

tion and not devoid of danger, as venous thrombosis may readily ensue. In the following experiments glucose was therefore given by the mouth, dissolved in water, just as in an ordinary tolerance test.

Some German authors, such as Nonnenbruch and Szyszka,<sup>48</sup> and Rosenberg,<sup>49</sup> assert that glucose injected intravenously induces glycosuria at a much lower blood sugar concentration than when it is given by the mouth, from which they conclude that the liver transforms the sugar in the latter case so that it passes with more difficulty through the kidneys. To test the validity of this statement, a number of experiments in which glucose was injected intravenously were made in connection with the experiments on the blood sugar and urine sugar after oral administration of glucose.

*Experiment No. 73.*

Person of experiment No. 3.

8 a. m.: 50 gm. glucose and 200 cc. water.

	Urine cc.	Benedict	Total sugar pro mil.	mg.	Blood sugar
7 a. m. to 8 a. m.	30	—	1.30	39	8 a. m. : 87
8.15 a. m.	14	—	0.19	39	8.15 " : 119
8.30 "	14	—			8.30 " : 161
8.45 "	70	—			8.45 " : 161
9 "	110	—			9 " : 151
9.15 "	196	—	0.09	38	9.15 " : 143
9.30 "	130	—			9.30 " : 98
9.45 "	64	—			9.45 " : 71
10 "	30	—			10 " : 71

*Experiment No. 74.*

Person of experiment No. 11.

9 a. m.: 50 gm. glucose and 200 cc. water.

	Urine cc.	Bene- dict	Total sugar pro mil.	mg.	Fermented pro mil.	mg.	Blood sugar
7 a. m. to 9 a. m.	220	—	0.26	57	0.10	22	9 a. m. : 90
							9.30 " : 172
9 " " 11 "	200	—	0.26	52	0.10	20	10 " : 161
							10.30 " : 130
							11 " : 105



*Experiment No. 75.*

Person of experiment No. 11.

9 a. m.: 80 gm. glucose and 200 cc. water.

		Urine Bene-		Total sugar		Fermented		Blood sugar
		cc.	dict	pro mil.	mg.	pro mil.	mg.	
7 a. m. to	9 a. m.	160	—	0.26	42	0.11	18	9 a. m. : 90
								9.30 " : 141
9 "	" 11 "	100	—	0.57	57	0.27	27	10 " : 183
								10.30 " : 152
								11 " : 143

*Experiment No. 76.*

Person of experiment No. 15.

4 p. m.: 50 gm. glucose and 200 cc. water.

		Urine Bene-		Total sugar		Fermented		Blood sugar
		cc.	dict	pro mil.	mg.	pro mil.	mg.	
3.30 to 4	p. m.	12	—	1.48	18	0.68	8	4 p. m. : 90
4 "	4.30 "	12	—	1.50	18	0.66	8	4.30 " : 132
4.30 "	5 "	15	+	1.80	27	1.11	17	5 " : 147
5 "	5.30 "	11	—	1.44	16	0.75	8	5.30 " : 112
5.30 "	6 "	10	—	1.28	13	0.68	7	6 " : 84

*Experiment No. 79.*

Person of experiment No. 18.

3 p. m.: 200 cc. water; 4. p. m.: 50 gm. glucose and 200 cc. water.

		Urine Bene-		Total sugar		Fermented		Blood sugar
		cc.	dict	pro mil.	mg.	pro mil.	mg.	
3 to 4	p. m.	120	—	0.30	36	0.15	18	4 p. m. : 70
								4.15 " : 126
4 "	4.30 "	195	—	0.45	88	0.28	55	4.30 " : 140
								4.45 " : 150
4.30 "	5 "	300	+	2.50	750	2.30	690	5 " : 126
								5.15 " : 88
5 "	5.30 "	65	+	2.10	137	1.90	124	5.30 " : 80

*Experiment No. 80.*

Person of experiment No. 18.

4 p. m.: 50 gm. glucose and 200 cc. water.

		Urine Bene-		Total sugar		Fermented		Blood sugar
		cc.	dict	pro mil.	mg.	pro mil.	mg.	
3 to 4	p. m. :	85	—	0.40	34	0.18	16	4 p. m. : 88
								4.15 " : 92
4 "	4.30 " :	25	—	0.70	18	0.57	14	4.30 " : 145
								4.45 " : 166
4.30 "	5 " :	26	+	1.77	46	1.61	42	5 " : 185
								5.15 " : 194
5 "	5.30 " :	21	+	3.75	79	3.55	75	5.30 " : 145
								5.45 " : 120
5.30 "	6 " :	19	—	1.16	22	0.91	17	6 " : 90

*Experiment No. 81.*

Person of experiment No. 19.

4 p. m.: 50 gm. glucose and 200 cc. water.

			Urine cc.	Bene- dict	Total sugar pro mil.	sugar mg.	Fermented pro mil.	mg.	Blood sugar
3	to 4	p. m. :	50	—	0.60	30	0.30	15	4 p. m. : 98
									4.15 " : 131
4	" 4.30	" :	23	—	0.90	21	0.35	12	4.30 " : 145
									4.45 " : 154
4.30	" 5	" :	19	—	1.16	22	0.40	15	5 " : 143
									5.15 " : 131
5	" 5.30	" :	17	—	1.12	19	0.40	13	5.30 " : 115
									5.45 " : 98
5.30	" 6	" :	16	—	0.96	15	0.30	9	

*Experiment No. 82.*

Person of experiment No. 19.

4 p. m.: 50 gm. glucose and 1,000 cc. water.

			Urine cc.	Bene- dict	Total sugar pro mil.	sugar mg.	Fermented pro mil.	mg.	Blood sugar
3	to 4	p. m. :	60	—	0.42	26	0.20	12	4 p. m. : 102
									4.15 " : 140
4	" 4.30	" :	26	—	0.66	17	0.44	11	4.30 " : 145
									4.45 " : 140
4.30	" 5	" :	160	—	0.22	35	0.07	20	5 " : 130
									5.15 " : 119
5	" 5.30	" :	340	—	0.11	37	0.03	20	5.30 " : 105
									5.45 " : 95
5.30	" 6	" :	250	—	0.14	35	0.03	17	

*Experiment No. 83.*

Person of experiment No. 21.

4 p. m.: 50 gm. glucose and 200 cc. water.

			Urine		Blood sugar		
			cc.	Benedict			
3.30 to 4	p. m.	:	25	—	4	p. m.	: 103
					4.15	"	: 123
4	" 4.30	"	: 17	—	4.30	"	: 160
					4.45	"	: 183
4.30	" 5	"	: 15	—	5	"	: 164
					5.15	"	: 132
5	" 5.30	"	: 15	—	5.30	"	: 97
					5.45	"	: 80
5.30	" 6	"	: 21				

*Experiment No. 84.*

Person of experiment No. 22.

8 a. m.: 50 gm. glucose and 200 cc. water.

## Blood sugar

8	a.m.	:	90	
8.15	"	:	120	
8.30	"	:	145	
8.45	"	:	149	
9	"	:	138	
9.15	"	:	124	
9.30	"	:	112	
9.45	"	:	100	
10	"	:	92	

Benedict's reaction negative  
in the urines passed every  
15 minutes between 8 and 10.

*Experiment No. 85.*

Person of experiment No. 23.

4 p. m.: 50 gm. glucose and 200 cc. water.

## Blood sugar

4	p.m.	:	95	
4.15	"	:	155	
4.30	"	:	164	
4.45	"	:	157	
5	"	:	155	
5.15	"	:	130	
5.30	"	:	95	
5.45	"	:	80	

Benedict's reaction negative.

*Experiment No. 86.*

Person of experiment No. 24.

8 a. m.: 50 gm. glucose and 200 cc. water.

			Urine		Total sugar			
			cc.	Benedict	pro mil.	mg.	Blood sugar	
7.30 to 8	a.m.		40	—	0.46	18	8	a.m. : 81
							8.15	" : 114
8	"	8.30	29	—	0.57	17	8.30	" : 126
							8.45	" : 154
8.30	"	9	16	—	0.85	14	9	" : 160
							9.15	" : 156
9	"	9.30	28	—	0.62	17	9.30	" : 147
							9.45	" : 129
9.30	"	10	105	—	0.20	21	10	" : 125



*Experiment No. 87.*

Person of experiment No. 24.

8 a. m.: 100 gm. glucose and 200 cc. water.

			Urine cc.	Benedict	Total sugar pro mil.	mg.	Blood sugar
7.30 to 8	a. m.	:	40	—	0.46	18	8 a. m. : 81
							8.15 " : 114
8	" 8.30	" :	29	—	0.57	17	8.30 " : 126
							8.45 " : 154
8.30	" 9	" :	16	—	0.85	14	9 " : 169
							9.15 " : 156
9	" 9.30	" :	28	—	0.62	17	9.30 " : 147
							9.45 " : 129
9.30	" 10	" :	103	—	0.20	21	10 " : 125

*Experiment No. 88.*

Person of experiment No. 25.

4 a. m.: 50 gm. glucose and 200 cc. water.

			Urine cc.	Benedict	Total sugar pro mil.	mg.	Blood sugar
3.30 to 4	p. m.	:	30	—	0.55	17	4 p. m. : 90
							4.15 " : 132
4	" 4.30	" :	30	—	0.73	22	4.30 " : 143
							4.45 " : 150
4.30	" 5	" :	40	—	0.60	24	5 " : 134
							5.15 " : 121
5	" 5.30	" :	155	—	0.15	23	5.30 " : 95
							5.45 " : 80
5.30	" 6	" :	155	—	0.15	23	

*Experiment No. 89.*

Person of experiment No. 27.

8 a. m.: 50 gm. glucose and 200 cc. water.

			Urine cc.	Bene- dict	Total sugar pro mil.	mg.	Fermented pro mil.	mg.	Blood sugar
7 a. m. to 8	a. m.	:	34	—	0.57	19	0.29	10	8 a. m. : 89
	8.15	" :	9	—	0.65	21	0.21	11	8.15 " : 124
	8.30	" :	5	—					8.30 " : 138
	8.45	" :	9	—					8.45 " : 143
	9	" :	9	—					9 " : 154
	9.15	" :	10	—	0.45	18	0.25	10	9.15 " : 138
	9.30	" :	12	—					9.30 " : 124
	9.45	" :	12	—					9.45 " : 100
	10	" :	5	—					10 " : 93

*Experiment No. 90.*

Person of experiment No. 29.

8 a. m.: 50 gm. glucose and 200 cc. water.

		Urine		
		cc.	Benedict	Blood sugar
7 a. m. to 8	a. m. :	30	—	90
8.15	" :	10	—	111
8.30	" :	10	—	150
8.45	" :	24	+	196
9	" :	55	—	134
9.15	" :	60	—	108
9.30	" :	23	—	83
9.45	" :	15	—	78
10	" :	12	—	67

*Experiment No. 91.*

Person of experiment No. 29.

8 a. m.: 50 gm. glucose and 200 cc. water.

		Urine		Total sugar		Blood sugar	
		cc.	Benedict	pro mil.	mg.		
7 a. m. to 8	a. m.	: 53	—	0.50	27	8 a. m.	: 92
8.15	"	: 10	—	0.45	31	8.15	" : 114
8.30	"	: 8	—			8.30	" : 149
8.45	"	: 14	—			8.45	" : 143
9	"	: 37	—			9	" : 92
9.15	"	: 50	—	0.15	27	9.15	" : 85
9.30	"	: 50	—			9.30	" : 85
9.45	"	: 55	—			9.45	" : 85
10	"	: 25	—			10	" : 89

*Experiment No. 92.*

Person of experiment No. 30

8 a. m.: 50 gm. glucose and 200 cc. water.

		Urine		
		cc.	Benedict	Blood sugar
8 a. m. to 8.15	a. m. :	14	—	89
8.30	" :	17	—	92
8.45	" :	110	—	110
9	" :	135	—	133
9.15	" :	130	—	147
9.30	" :	60	—	127
9.45	" :	35	—	108
10	" :	18	—	90

*Experiment No. 93.*

Person of experiment No. 30.

8 a. m.: 50 gm. glucose and 200 cc. water.

		Urine		Total sugar		Blood sugar	
		cc.	Benedict	pro mil.	mg.		
7 a. m. to 8	a. m. :	35	—	0.66	23	8 a. m. :	97
8.15	" :	13	—	0.15	30	8.15	" : 163
8.30	" :	17	—			8.30	" : 171
8.45	" :	65	—			8.45	" : 130
9	" :	105	—			9	" : 124
9.15	" :	50	—	0.15	21	9.15	" : 116
9.30	" :	37	—			9.30	" : 101
9.45	" :	30	—			9.45	" : 80
10	" :	22	—			10	" : 72

*Experiment No. 94.*

Person of experiment No. 31.

8 a. m.: 50 gm. glucose and 200 cc. water.

		Urine Bene-		Total sugar		Fermented		Blood sugar	
		cc.	dict	pro mil.	mg.	pro mil.	mg.		
7 a. m. to 8	a. m. :	75	—	0.32	24	0.12	9	8 a. m. :	97
8.15	" :	20	—	0.51	28	0.21	12	8.15	" : 160
8.30	" :	12	—					8.30	" : 206
8.45	" :	11	—					8.45	" : 156
9	" :	12	—					9	" : 151
9.15	" :	12	—	0.40	31	0.07	6	9.15	" : 120
9.30	" :	13	—					9.30	" : 109
9.45	" :	23	—					9.45	" : 90
10	" :	29	—					10	" : 91

*Experiment No. 95.*

Person of experiment No. 33

		Urine Bene-		Total sugar		Fermented		Blood sugar	
		cc.	dict	pro mil.	mg.	pro mil.	mg.		
8 a. m. to 9	a. m. :	27	—	0.90	24	0.35	9	9 a. m. :	88
9.15	" :	13	—	0.30	27	0.13	12	9.15	" : 88
9.30	" :	8	—					9.30	" : 110
9.45	" :	30	—					9.45	" : 127
10	" :	40	—					10	" : 147
10.15	" :	42	—	0.20	27	0.08	12	10.15	" : 127
10.30	" :	46	—					10.30	" : 96
10.45	" :	34	—					10.45	" : 90
11	" :	11	—					11	" : 88



*Experiment No. 96.*

Person of experiment No. 34.

8 a. m.: 50 gm. glucose and 200 cc. water.

		Urine		Total sugar				Blood sugar
		cc.	Benedict	pro mil.	mg.			
7 a. m. to 8	a. m.	60	—	0.62	37		8 a. m. :	95
8.15	"	15	—	0.50	38		8.15 "	: 135
8.30	"	15	—				8.30 "	: 150
8.45	"	15	—				8.45 "	: 121
9	"	30	—				9 "	: 109
9.15	"	42	—	0.35	39		9.15 "	: 95
9.30	"	22	—				9.30 "	: 90
9.45	"	33	—				9.45 "	: 88
10	"	15	—				10 "	: 87

*Experiment No. 97.*

Person of experiment No. 35.

8 a. m.: 50 gm. glucose and 200 cc. water.

		Urine	Bene-	Total sugar		Fermented		Blood sugar
		cc.	dict	pro mil.	mg.	pro mil.	mg.	
7 a. m. to 8	a. m.	22	—	0.89	20	0.35	8	8 a. m. : 90
8.15	"	7	—	2.52	86	1.80	61	8.15 " : 145
8.30	"	7	—					8.30 " : 170
8.45	"	7	+					8.45 " : 200
9	"	13	+					9 " : 201
9.15	"	16	+	2.40	118	1.90	93	9.15 " : 168
9.30	"	13	+					9.30 " : 135
9.45	"	9	—					9.45 " : 110
10	"	11	—					10 " : 92

*Experiment No. 98.*

Person of experiment No. 36.

8 a. m.: 50 gm. glucose and 200 cc. water.

		Urine	Bene-	Total sugar		Fermented		Blood sugar
		cc.	dict	pro mil.	mg.	pro mil.	mg.	
7 a. m. to 8	a. m.	34	—	0.66	22	0.30	10	8 a. m. : 93
8.15	"	8	—	0.75	23	0.27	8	8.15 " : 120
8.30	"	7	—					8.30 " : 145
8.45	"	8	—					8.45 " : 162
9	"	7	—					9 " : 170
9.15	"	10	—	0.75	28	0.30	11	9.15 " : 135
9.30	"	8	—					9.30 " : 92
9.45	"	12	—					9.45 " : 89
10	"	8	—					10 " : 88

Experiment No. 99.

Person of experiment No. 37.

8 a. m.: 50 gm. glucose and 200 cc. water.

		Urine	Bene-	Total sugar		Fermented		Blood sugar
		cc.	dict	pro mil.	mg.	pro mil.	mg.	
7 a. m. to 8	a. m.	66	—	0.35	23	0.12	8	8 a. m. : 86
8.15	"	9	—	0.28	53	0.10	19	8.15 " : 107
8.30	"	8	—					8.30 " : 137
8.45	"	37	—					8.45 " : 144
9	"	135	—					9 " : 134
9.15	"	91	—	0.32	44	0.14	19	9.15 " : 125
9.30	"	20	—					9.30 " : 114
9.45	"	14	—					9.45 " : 97
10	"	14	—					10 " : 82

Experiment No. 100.

Person of experiment No. 40.

8 a. m.: 50 gm. glucose and 200 cc. water.

		Urine	Benedict	Total sugar		Blood sugar
		cc.		pro mil.	mg.	
7 a. m. to 8	a. m.	43	—	0.50	22	8 a. m. : 98
8.15	"	10	—	0.78	23	8.15 " : 133
8.30	"	4.5	—			8.30 " : 163
8.45	"	6	—			8.45 " : 176
9	"	8	—			9 " : 138
9.15	"	5	—	1.0	24	9.15 " : 105
9.30	"	7	—			9.30 " : 95
9.45	"	6	—			9.45 " : 90
10	"	6	—			10 " : 80

Experiment No. 101.

Person of experiment No. 41.

8 a. m.: 50 gm. glucose and 200 cc. water.

		Urine	Bene-	Total sugar		Fermented		Blood sugar
		cc.	dict	pro mil.	mg.	pro mil.	mg.	
7 a. m. to 8	a. m.	40	—	0.45	18	0.20	8	8 a. m. : 105
8.15	"	7	—	0.88	26	0.65	19	8.15 " : 136
8.30	"	8.5	—					8.30 " : 171
8.45	"	7	—					8.45 " : 165
9	"	6.5	—					9 " : 160
9.15	"	9	—	0.45	32	0.27	19	9.15 " : 117
9.30	"	13	—					9.30 " : 103
9.45	"	29	—					9.45 " : 70
10	"	20	—					10 " : 50

*Experiment No. 102.*

Person of experiment No. 51.

8 a. m.: 50 gm. glucose and 200 cc. water.

	Urine cc.	Bene- dict	Total sugar pro mil.	sugar mg.	Fermented pro mil.	mg.	Blood sugar
7 a. m. to 8 a. m.	30	—	0.45	14	0.23	7	8 a. m. : 96
							8.15 " : 143
8 " " 9 "	17	—	0.84	14	0.45	8	8.30 " : 184
							8.45 " : 216
9 " " 10 "	17	—	1.0	17	0.59	10	9 " : 143
							9.15 " : 135
							9.30 " : 115
							9.45 " : 110
							10 " : 105

Among the persons of experiment were some medical students, who had lectures in the forenoon. They were, therefore, examined in the afternoon, 3 to 4 hours after the last meal. In the tables below, the experiments done in the afternoon are tabulated separately.

*Experiments in the morning, 12 to 14 hours after the last meal.*

A. 50 gm. of glucose and 200 cc. of water.

Experi- ment No.	Blood sugar				Benedict	Sugar in Urine
	Before	Maxi- mum	Maximum after	Again below 110 mg.		
73	87	161	½ and ¾ hrs.	1½ hrs.	—	unchanged
74	90	172	½ hr.	2 "	—	unchanged
84	90	149	¾ "	1½ "	—	not tested
86	90	160	1 "	1¼ "	—	not tested
89	89	154	1 "	1¾ "	—	unchanged
90	90	196	¾ "	1¼ "	+	increased
91	92	149	½ "	1 "	—	unchanged
92	89	147	1 "	1½ "	—	not tested
93	97	171	½ "	1½ "	—	increased
94	97	206	½ "	1½ "	—	increased
95	88	147	¾ "	1½ "	—	unchanged
96	95	150	½ "	1 "	—	unchanged
97	90	201	1 "	2 "	+	increased
98	93	170	1 "	1½ "	—	unchanged
99	86	144	¾ "	1¾ "	—	increased
100	98	176	¾ "	1¼ "	—	unchanged
101	105	171	½ "	1½ "	—	increased
102	96	216	¾ "	2 "	—	unchanged



## B. More than 50 gm. of glucose.

80 gm. glucose and 200 cc. water.

Experiment No.	———Blood sugar———					Sugar in Urine
	Before	Maxi- mum	Maximum after	Again below 110 mg.	Benedict	
75	90	183	1 hr.	after 2 hrs. 143 mg.	—	increased

100 gm. glucose and 200 cc. water.

Experiment No.	———Blood sugar———					Sugar in Urine
	Before	Maxi- mum	Maximum after	Again below 110 mg.	Benedict	
87	81	160	1 hr.	after 2 hrs. 125	—	unchanged

*Experiments in the afternoon, 4 to 5 hours after the last meal.*

## A. 50 gm. of glucose and 200 cc. of water.

Experiment No.	———Blood sugar———					Sugar in Urine
	Before	Maxi- mum	Maximum after	Again below 110 mg.	Benedict	
76	90	147	1 hr.	2 hrs.	+	
79	70	150	$\frac{3}{4}$ "	$1\frac{1}{4}$ "	+	increased
80	88	194	$1\frac{1}{4}$ "	2 "	+	increased
81	98	154	$\frac{3}{4}$ "	2 "	—	increased
83	103	183	$\frac{3}{4}$ "	$1\frac{1}{2}$ "	—	increased
85	95	164	$\frac{1}{2}$ "	$1\frac{1}{2}$ "	—	
88	90	150	$\frac{3}{4}$ "	$1\frac{1}{2}$ "	—	unchanged

## B. More than 200 cc. of water.

50 gm. glucose and 600 cc. water.

Experiment No.	———Blood sugar———					Sugar in Urine
	Before	Maxi- mum	Maximum after	Again below 110 mg.	Benedict	
78	80	147	$\frac{3}{4}$ hr.	2 hrs.	+	increased

50 gm. glucose and 1,000 cc. water.

Experiment No.	———Blood sugar———					Sugar in Urine
	Before	Maxi- mum	Maximum after	Again below 110 mg.	Benedict	
81	102	145	$\frac{1}{2}$ hr.	$1\frac{1}{2}$ hrs.	—	increased

In the experiments recorded in the above tables, urine and blood samples, when not otherwise stated, were taken simulta-

neously before the experiment was started, and every 15 minutes for 2 hours after the administration of the sugar solution.

Thirty experiments were made on 23 persons, 7 of the latter being used on 2 occasions.

*The blood sugar concentration* just before starting the experiments in the morning varied between 81 and 105, the mean being 90 mg. per 100 cc. blood (20 estimations), and in the afternoon, between 70 and 107, the mean being 93 (9 estimations).

*The maximal* blood sugar concentration varied in the morning experiments, after 50 gm. glucose dissolved in 200 cc. water, between 144 and 216 mg., the mean being 169 (18 experiments), while the 2 experiments with 80 and 100 gm. glucose, respectively, had maximal blood sugar concentrations of 183 and 160, the mean being 171.5.

In the experiments in the afternoon with 50 gm. glucose dissolved in 200 cc. water, the blood sugar rise varied between 147 and 194 mg., with a mean of 163 (7 experiments).

After the 2 experiments with large quantities of water, the greatest blood sugar concentrations were 147 and 145 mg. respectively, the mean therefore being 146.

The maximal blood sugar concentration occurred most frequently in  $\frac{3}{4}$  hour (10 times), then about an equal number of times in half an hour and one hour. Once, in experiment No. 80, the highest value was only reached on  $1\frac{1}{4}$  hours.

In all the experiments with 50 gm. glucose (27) the blood sugar had again fallen below 110 in 2 hours, and in 16 of the 27 experiments it was less than 110 before  $1\frac{1}{2}$  hours had elapsed. On the other hand in the 2 experiments with 80 and 100 gm. glucose, it was greater than 110 after the lapse of 2 hours.

Three persons received 50 gm. glucose dissolved in 200 cc. water for 2 doses. If the courses of the blood sugar curves for both administrations are compared it will be seen that in case 18 (experiments 79 and 80) the blood sugar rise in experiment 80 was much greater and of longer duration than in experiment 79. An hour and a quarter after the experiment was started the blood sugar concentration in experiment 79 was therefore 80 mg., while in the other experiment, done under precisely the same conditions, it was 194.

In experiments 90 and 91 (case No. 29), the blood sugar curves also showed a somewhat different course, the maximal blood sugar concentration in one experiment being 196, and in the other only 149.

In experiments 92 and 93, there was again a difference, although not so large as in the foregoing experiment, the figures for the greatest blood sugar values being 147 and 171, respectively.

Benedict's qualitative sugar reaction in the morning experiments with 50 gm. glucose and 200 cc. water was twice positive (experiments 90 and 97) out of 18 experiments, while in the afternoon experiments it was positive 3 times out of 7.

In the 2 experiments with 80 and 100 gm. glucose, respectively, there was no clinically demonstrable glycosuria.

The urine sugar estimated by Benedict and Osterberg's method, with the slight modification previously mentioned, was of course considerably increased in the experiments where Benedict's qualitative reaction was positive, but it was also increased in 4 experiments, Nos. 99, 101, 75 and 81, when the reaction was not positive. In 2 experiments, Nos. 93 and 94, the increase was so slight that it must be regarded as doubtful. In all the other experiments in which the blood sugar concentration varied between 87 and 216 mg. per 100 cc., no change in the amount of urine sugar was found during the experiments.

For determination of the *renal threshold* experiments with the administration of large amounts of glucose are not well adapted, as Faber and Norgaard<sup>36</sup> have pointed out, since the blood sugar curve is generally too steep. The position of the threshold can therefore only be located within rather wide limits in these experiments. The threshold determination is also inaccurate because the person has to urinate very frequently,—every 15 minutes in such experiments,—and thus even a trace of urine remaining in the bladder after urination may affect the result by contaminating the sample excreted during the succeeding period of the experiment.

From the experiments which were unaccompanied by clinical glycosuria or a marked increase in the urine sugar, it can only be concluded that the threshold was higher than the maximal blood sugar rise, which varied between 147 and 216. In the cases that got clinically demonstrable glycosuria the renal threshold was situated between the following boundaries:



		Calculated from the increasing part of the curve	Calculated from the decreasing part of the curve
Experiment No. 97	:	170 to 200	168 to 135
" " 90	:	150 " 196	
" " 76	:	132 " 147	
" " 78	:	80 " 140	
" " 79	:	140 " 150	126 " 80
" " 80	:	145 " 185	185 " 145

Experiments 79 and 80 refer to the same person. In experiment 80 no glycosuria was detected while the blood sugar fell from 145 to 79, but in experiment 79 it was present between 126 and 80, as is evident from the table.

As will be observed, the threshold varied unusually much, the lowest value being less than 140, while the highest was more than 216. The investigations further prove that the threshold may vary during the experiments, for it may be lower during the falling than the rising portion of the blood sugar curve, and, as experiments 79 and 80 seem to show, it may also vary during the falling part of the blood sugar curve in uniform experiments in the same person.

*The Renal Threshold With Intravenous Injection of Glucose.*

In connection with these experiments on the administration of glucose by the mouth, a number of experiments were performed, as stated above, on the intravenous injection of glucose to gain some knowledge of the situation of the threshold in such a case. Merck's chemically pure water-free glucose in a 50% solution in water was used, of which 10-20 cc. was injected, that is, 5-10 gm. glucose in an arm vein. Two patients with heart disease were employed for the experiment.

*Case No. 58.* The patient felt comparatively well. Apart from slight edema of the legs there were no signs of lack of compensation.

*Experiment No. 103.*

November 3, 1921, 20 cc. of 50 per cent. glucose solution was injected intravenously before breakfast.

Blood sugar before

the injection: 87 mg.

5 minutes later : 163

15 " " : 140

30 " " : 124

60 " " : 103

90 " " : 103

The urine was examined before the injection, 30 minutes, 60 minutes and 90 minutes after the injection. None of the samples contained sugar.

*Experiment No. 104.*

November 4th, the same patient was injected with the same amount of glucose.

Blood sugar before the injection :	92 mg.	
5 minutes later :	196 "	
15 " " :	142 "	No glycosuria.
30 " " :	126 "	
45 " " :	107 "	
60 " " :	103 "	

*Experiment No. 105.*

November 5th, the injection was repeated in the same patient.

Blood sugar before the injection :	83 mg.	
3 minutes later :	179 "	
6 " " :	152 "	No glycosuria.
9 " " :	148 "	
12 " " :	142 "	
15 " " :	131 "	

*Case No. 59.* The patient had had heart disease for a long time, but was out of bed and, apart from some cyanosis, showed no signs of lack of compensation.

*Experiment No. 106.*

November 3rd, 10 cc. of 50 per cent. glucose solution intravenously before breakfast.

Blood sugar before the injection :	90 mg.	
5 minutes later :	145 "	
15 " " :	143 "	No glycosuria.
30 " " :	126 "	
45 " " :	125 "	
60 " " :	121 "	

By comparing these experiments with those relating to the oral administration of glucose, it will be noticed that the amount injected intravenously constituted only 1/5-1/10 of the amount given by the mouth. Nevertheless, the maximal blood sugar rise was about the same in the two series of experiments. But the maximal blood sugar concentration is reached on intravenous injection—as would be expected—much earlier than when the glucose is taken by the mouth. In experiment 105, as will be seen, the maximal blood sugar rise is already reached in 3 minutes. Furthermore, the blood sugar curve falls much more quickly than by oral administration, so that the greater part of the hyperglycemia has disappeared in half an hour's time.

As glycosuria was not present in these cases the situation of the threshold cannot be determined, but it can be concluded from the experiments that it does not lie very low with intravenous injection of glucose, because the blood sugar rise in the one patient reached 145, and in the other, 163, 196 and 179, in the 3 experiments without clinically demonstrable glycosuria making its appearance.

*The Effect on the Urine Sugar and Blood Sugar of the Administration of Glucose, together with Acids, Alkalis, or Food.*

As already mentioned in the introductory remarks, Benedict and his pupils<sup>19</sup> assert that during digestion the organism's sugar tolerance is depressed presumably because the "external function" of the pancreas, that is to say its digestive work, depresses its "internal function." In order to further investigate this question, a number of persons whose tolerance had previously been tested with glucose and water, were given glucose and bread, and in one experiment meat also, after which the blood sugar and urine sugar were estimated as usual. In addition an experiment in which HCl and glucose were given simultaneously was made, and also one with glucose and sodium bicarbonate.

*Experiment No. 107.*

Person of experiment No. 41.

8 a. m.: 50 gm. glucose + 2 cc. dilute HCl + 200 cc. water.

	Urine cc.	Bene- dict	Total sugar pro mil.	mg.	Fermented pro mil.	mg.	Blood sugar
7 a. m. to 8 a. m.	32	—	0.70	22	0.30	10	8 a. m. : 100
8.15 a. m.	8	—	0.75	20	0.42	11	8.15 " : 112
8.30 "	6	—					8.30 " : 136
8.45 "	6.5	—					8.45 " : 124
9 "	5.5	—					9 " : 117
9.15 "	7	—	0.78	24	0.44	13	9.15 " : 102
9.30 "	5.5	—					9.30 " : 67
9.45 "	11.5	—					9.45 " : 55
10 "	6.5	—					10 " : 45



*Experiment No. 108.*

Person of experiment No. 41.

8 a. m.: 50 gm. glucose + 15 gm. soda bicarb. + 200 cc. water.

	Urine cc.	Benedict	Total sugar		Blood sugar
			pro mil.	mg.	
7 a. m. to 8 a. m.	34	—	0.70	24	8 a. m. : 95
8.15 a. m.	7.5	—	1.60	26	8.15 " : 120
8.30 "	2.5	—			8.30 " : 142
8.45 "	2.5	—			8.45 " : 130
9 "	4	—			9 " : 105
9.15 "	5	—	0.70	27	9.15 " : 80
9.30 "	9	—			9.30 " : 53
9.45 "	12	—			9.45 " : 53
10 "	12	—			10 " : 65

The last two experiments were done with the same person and they showed about the same blood sugar rise. The urine sugar was not changed in any of the experiments.

*Experiment No. 109.*

Person of experiment No. 51.

8 a. m.: 100 gm. bran bread + 50 gm. glucose + 200 cc. water.

	Urine cc.	Bene- dict	Total sugar		Fermented		Blood sugar
			pro mil.	mg.	pro mil.	mg.	
7 a. m. to 8 a. m.	18	—	0.53	10	0.33	6	8 a. m. : 93
							8.15 " : 130
8 " " 9 "	28	—	0.97	27	0.39	11	8.30 " : 154
							8.45 " : 163
9 " " 10 "	27	+	1.64	44	0.64	17	9 " : 115
							9.15 " : 110
10 " " 11 "	20	+	2.10	44	1.30	26	9.30 " : 108
							9.45 " : 108
							10 " : 105

*Experiment No. 110.*

Person of experiment No. 22.

8 a. m.: 100 gm. rye bread + 50 gm. glucose + 200 cc. water.

	Urine cc.	Bene- dict	Total sugar		Fermented		Blood sugar
			pro mil.	mg.	pro mil.	mg.	
7 a. m. to 8 a. m.	50	—	0.45	23	0.20	10	8 a. m. : 94
8.15 a. m.	13	—					8.15 " : 125
8.30 "	10	—					8.30 " : 151
8.45 "	7	—	0.85	35	0.32	13	8.45 " : 145
9 "	11	—					9 " : 114
9.15 "	6	—					9.15 " : 96
9.30 "	10	—	1.35	38	0.50	14	9.30 " : 94
9.45 "	6	—					9.45 " : 95
10 "	6	—					10 " : 92

*Experiment No. 111.*

Person of experiment No. 22.

8 a. m.: 100 gm. bran bread + 50 gm. glucose + 200 cc. water.

	Urine cc.	Benedict	Total sugar		Blood sugar
			pro mil.	mg.	
7 a. m. to 8 a. m.	80	—	0.34	27	8 a. m. : 94
8.30 a. m.	45	—	0.32	22	8.15 " : 130
8.45 "	15	—			8.30 " : 179
9 "	10	—			8.45 " : 150
9.15 "	11	—			9 " : 123
9.30 "	12	—	1.20	52	9.15 " : 94
9.45 "	11	—			9.30 " : 84
10 "	9	—			9.45 " : 83
10.15 "	8	+			10 " : 84
10.30 "	10	+	1.40	53	10.15 " : 88
10.45 "	13	—			10.30 " : 91
11 "	7	—			11 " : 94

*Experiment No. 112.*

Person of experiment No. 33.

9 a. m.: 100 gm. bran bread and 200 cc. water.

10 a. m.: 50 gm. glucose and 200 cc. water.

	Urine cc.	Bene- dict	Total sugar		Fermented		Blood sugar
			pro mil.	mg.	pro mil.	mg.	
8 a. m. to 9 a. m.	40	—	0.75	30	0.35	14	
9.30 a. m.	21	—	1.05	43	0.48	20	
10 "	20	+					10 a. m. : 104
10.30 "	18	+	1.31	54	0.65	27	10.15 " : 111
11 "	23	+					10.30 " : 141
11.30 "	49	—	0.98	74	0.42	30	10.45 " : 147
12 m.	22	—					11 " : 113
							11.15 " : 96
							11.30 " : 87
							11.45 " : 80
							12 m. : 78

*Experiment No. 113.*

Person of experiment No. 31.

8 a. m.: 100 gm. bran bread and 200 cc. water.

10 a. m.: 50 gm. glucose and 200 cc. water.

	Urine cc.	Benedict	Total sugar		Blood sugar
			pro mil.	mg.	
7 a. m. to 8 a. m.	66	—	0.35	25	8 a. m. : 85
8.30 a. m.	41	—	0.54	37	8.30 " : 85
8.45 "	15	—			
9 "	13	—			9 " : 86
9.15 "	19	—			
9.30 "	15	—	1.14	66	9.30 " : 87
9.45 "	13	—			
10 "	11	—			10 " : 86
10.15 "	14	—			10.15 " : 133
10.30 "	10	—	1.12	52	10.30 " : 156
10.45 "	10	—			10.45 " : 114
11 "	12	—			11 " : 84
11.15 "	17	—			11.15 " : 80
11.30 "	15	—	0.66	40	11.30 " : 85
11.45 "	14	—			
12 m.	14	—			

*Experiment No. 114.*

Person of experiment No. 35.

8 a. m.: 100 gm. bran bread and 200 cc. water.

10 a. m.: 50 gm. glucose and 200 cc. water.

	Urine cc.	Bene- dict	Total sugar		Fermented		Blood sugar
			pro mil.	mg.	pro mil.	mg.	
7 a. m. to 8 a. m.	30	—	1.27	38	0.50	15	
8 " " 9 "	35	—	1.42	50	0.60	21	9 a. m. : 115
9 " " 10 "	22	+	2.80	62	2.05	44	10 " : 95
10 " " 11 "	34	+	3.60	122	2.50	85	10.45 " : 194
							11 " : 143



*Experiment No. 115.*

Person of experiment No. 40.

8 a. m.: 300 gm. meat and 200 cc. water.

9 a. m.: 50 gm. glucose and 200 cc. water.

	Urine cc.	Bene- dict	Total sugar pro mil.	sugar mg.	Fermented pro mil.	mg.	Blood sugar
7 a. m. to 8 a. m.	40	—	0.51	20	0.25	10	
8 " " 9 "	35	—	0.60	21	0.28	10	9 a. m. : 100
9.15 a. m.	8.5	—	1.0	28.5	0.56	16	9.15 " : 153
9.30 "	5.5	—					9.30 " : 165
9.45 "	7.5	—					9.45 " : 162
10 "	7	—					10 " : 140
10.15 "	8	—	0.96	30	0.46	14	10.15 " : 102
10.30 "	7	—					10.30 " : 83
10.45 "	8	—					10.45 " : 74
11 "	8.5	—					11 " : 81

In the following table the hyperglycemia in the last two experiments is compared with the hyperglycemia in the same persons after glucose and water only.

Person of experiment No.	Experiment No.	Test meal	Blood sugar
51	102	Glucose and water	Greatest rise after glucose and water
	109	Bran bread, glucose and water	
	84	Glucose and water	
22	110	Rye bread, glucose and water	About the same rise in both experiments
	94	Glucose and water	
	111	Bran bread, glucose and water	
31	113	Bran bread, glucose and water	Greatest rise after glucose and water
	95	Glucose and water	
	112	Bran bread, glucose and water	
33	97	Glucose and water	About the same rise in both experiments
	114	Bran bread, glucose and water	
35			About the same rise in both experiments

Person of experiment No.	Experiment No.	Test meal	Blood sugar
40	100	Glucose and water	About the same rise in both experiments
	115	Meat, glucose and water	

It is evident from the table that bread or meat in addition to the glucose does not increase the blood sugar rise.

In the table below the sugar output after bread and meat is compared with the output following the ingestion of glucose together with bread and meat.

Person of experiment No.	Experiment No.	Test meal	Urine sugar
51	66	Bran bread and water	About the same rise in both experiments
	109	Glucose, bran bread and water	
22	45	Rye bread and water	About the same rise in both experiments
	110	Glucose, rye bread and water	
31	49	Bran bread and water	About the same rise in both experiments
	111	Glucose, bran bread and water	
33	50	Bran bread and water	About the same rise in both experiments
	112	Glucose, bran bread and water	
35	54	Bran bread and water	Greatest rise after glucose, the renal threshold being passed
	114	Glucose, bran bread and water	
40	69	Meat and water	About the same rise in both experiments
	115	Glucose, meat and water	

As will be observed from the table, the blood sugar after the administration of glucose in case No. 35 rose above the threshold, causing a considerable increase in the sugar excretion. In all the other five parallel experiments, glucose in association with bread or meat did not alter the amount of urine sugar in the slightest degree.

The independence of the physiological urine sugar of changes in the blood sugar concentration was demonstrated very clearly in these experiments. In experiments 109, 110 and 111, glucose was given together with bread which led to considerable hyperglycemia in the first hour, but the amount of urine sugar was unchanged or only slightly increased. In the second and third hours, however, the blood sugar had fallen to normal, while the urine sugar rose a good deal.

### III. *Transition Cases.*

Since Klemperer<sup>50</sup> described "renal diabetes" in 1896, numerous cases of glycosuria have been published which with more or less justification have been regarded as innocent and which have been given various designations, such as renal glycosuria, renal diabetes, diabetes innocens, cyclic glycosuria, etc.

In including the following cases under the heading "transition cases," it is intended to convey that they have some symptoms in common with diabetes patients, but no opinion is expressed as to whether they are benign or not.

*Case No. 16.* Medical student, J., 24 years old; weight about 68 kg. No diabetes in the family. Never had any grave illness. Considers himself absolutely healthy. His glycosuria was discovered when he kindly volunteered for experiment.

#### *Experiment No. 77.*

4 p. m.: 50 gm. glucose and 200 cc. water.

	Urine cc.	Bene- dict	Total sugar pro mil.	sugar mg.	Fermented pro mil.	mg.	Blood sugar
3 p. m. to 4 p. m.	20	—	1.21	24	0.50	10	4 p. m. : 70
							4.15 " : 126
4.30 p. m.	20	+	6.85	137	6.0	120	4.30 " : 160
							4.45 " : 205
5 "	30	+	50.0	1500	49.3	1479	5 " : 150
							5.15 " : 126
5.30 "	17	+	12.50	213	12	204	5.30 " : 95
							5.45 " : 80
6 "	20	—	1.0	20	0.40	8	





*Experiment No. 117.*

9 a. m.: 50 gm. glucose and 200 cc. water.

	Urine cc.	Bene- dict	Total sugar pro mil.	mg.	Fermented pro mil.	mg.	Blood sugar
8 a. m. to 9 a. m.	28	—	1.20	34	0.70	20	9 a. m. : 96
9.15 a. m.	7	—	1.45	38	1.0	26	9.15 " : 107
9.30 "	6	—					9.30 " : 129
9.45 "	7	—					9.45 " : 126
10 "	6	—					10 " : 114
10.15 "	7	—	1.05	34	0.60	19	10.15 " : 100
10.30 "	8	—					10.30 " : 83
10.45 "	8	—					10.45 " : 83
11 "	9	—					11 " : 83

*Experiment No. 118.*

8 a. m.: 100 gm. bran bread and 200 cc. water.

	Urine cc.	Bene- dict	Total sugar pro mil.	mg.	Fermented pro mil.	mg.	Blood sugar
8.30 to 9 a. m.	14	—	0.75	21	0.41	12	
9 " 9.45 "	23	—	0.80	18	0.46	11	9.45 a. m. : 99
9.45 " 10 "	10	+	1.90	46	0.96	24	10 " : 112
10 " 10.15 "	15	+					10.15 " : 99
10.15 " 10.30 "	20	—	1.14	23	0.76	15	10.30 " : 96
10.30 " 10.45 "	26	—	0.70	36	0.42	22	10.45 " : 94
10.45 " 11 "	26	—					

The determination of the urine sugar in unequal periods makes a comparison of the output in every thirty minutes difficult. It is, however, evident that both the total and the fermentable sugar increased after the bread.

*Experiment No. 119.*

9 a. m.: 100 gm. rye bread and 200 cc. water.

	Urine cc.	Benedict	Total sugar pro mil.	mg.	Blood sugar
8.30 to 9 a. m.	12	—	1.15	14	9 a. m. : 95
9.15 a. m.	5	—	1.15	13	9.15 " : 100
9.30 "	6	—			9.30 " : 108
9.45 "	6	—	1.84	22	9.45 " : 134
10 "	6	+			10 " : 115
10.15 "	8	—	1.35	24	10.15 " : 115
10.30 "	10	—			10.30 " : 90
10.45 "	13	—	0.85	23	10.45 " : 85
11 "	14	—			

From the 4 experiments on this person it appears that the fasting blood sugar in the morning was always normal. On two occasions 50 gm. glucose dissolved in 200 cc. water was given. On one of them, experiment 117, the blood sugar rose only to 129, and in  $1\frac{1}{4}$  hours it was again less than 110. Benedict's reaction was negative in all the urine samples, taken every 15 minutes. Simultaneously with the blood sugar rise there was a slight increase in the urine sugar which would indicate that the threshold had been exceeded by the blood sugar. In the 2nd experiment, No. 116, it rose to 158 in half an hour, and at the same time Benedict's reaction and the phenylhydrazin reaction became positive. In  $1\frac{3}{4}$  hours the blood sugar was again less than 110.

In experiment 118, it is doubtful whether the greater sugar excretion was due to the slight blood sugar rise or the brown bread as such (see experiment with brown bread). In experiment 119, the blood sugar rose to 134 after 100 gm. finely sifted rye bread, and simultaneously Benedict's reaction became positive.

As will be noticed, there was a low threshold in these cases situated between 131 and 158, but the fasting blood sugar in the morning was normal and there was a normal blood sugar rise after 50 gm. glucose.

*Person of experiment No. 20* was a medical student, 27 years old, weight 83.5 kg. He had never been ill and there was no diabetes in his family. His glycosuria was discovered by the experiments recorded below:

*Experiment No. 120.*

April 16th, 1920. 4 p. m.: 50 gm. glucose and 200 cc. water.

	Urine cc.	Total sugar pro mil.	mg.	Benedict	Blood sugar
3 to 4 p. m.	: 24	0.96	23	—	4 p. m. : 103
					4.15 " : 136
4.30 p. m.	: 15	1.50	25	+	4.30 " : 182
					4.45 " : 189
5 "	: 22	24.0	528	+	5 " : 150
					5.15 " : 121
5.30 "	: 16	3.0	48	+	5.30 " : 88
					5.45 " : 84
6 "	: 16	1.0	16	—	



*Experiment No. 121.*

December 13th, 1921. 4 p. m.: 50 gm. glucose and 200 cc. water.

	Urine cc.	Total sugar pro mil.	mg.	Benedict	Blood sugar
3 to 4 p. m.	: 30	1.0	30	—	4 p. m. : 90
					4.15 " : 100
4.30 p. m.	: 15	1.5	23	+	4.30 " : 114
					4.45 " : 141
5 "	: 12	8.0	96	+	5 " : 133
					5.15 " : 124
5.30 "	: 9	8.0	72	+	5.30 " : 115
6 "	: 9	2.0	18	+	6 " : 99

*Experiment No. 122.*

September 20th, 1922. 8 a. m.: 50 gm. glucose and 200 cc. water.

	Urine cc.	Benedict	Total sugar pro mil.	mg.	Fermented pro mil.	mg.	Blood sugar
7 to 8 a. m.	: 32	—	0.63	20	0.33	11	8 a. m. : 85
8.15 a. m.	: 10	—	4.13	194	3.77	177	8.15 " : 138
8.30 "	: 11	+					8.30 " : 158
8.45 "	: 11	+					8.45 " : 149
9 "	: 15	+					9 " : 134
9.15 "	: 30	+	0.38	64	0.27	45	9.15 " : 89
9.30 "	: 98	—					9.30 " : 60
9.45 "	: 20	—					9.45 " : 52
10 "	: 20	—					10 " : 69

Three tolerance tests were done in the course of 2½ years, each time with 50 gm. glucose and 200 cc. water. As he was decidedly stout at the time of the first investigation, he put himself on a limited diet and got considerably thinner, as appears from the notes of each test.

Before each of the three tests the blood sugar was normal, and the blood sugar curve fell below 110 in two hours in each case. The maximum blood sugar concentration varied a good deal in the 3 tests, but not more than is found in normal persons, as is evident from the previously discussed experiments.

The renal threshold in the three experiments was as follows:

Experiment No.	Blood sugar curve	
	Rising	Decreasing
120	Between 103 and 182	150 and 88
121	" 90 " 114	115 " 99
122	" 138 " 158	134 " 89

In experiment 122, there was no demonstrable glycosuria during the time the blood sugar rose from 85 to 138, but in experiment 121, it was

present while the blood sugar rose from 90 to 114. This seems to show that the position of the threshold may vary, as is also apparent from experiments with healthy individuals. It can further be concluded from the experiments that the threshold in this person is very low and that this is the only anomaly that can be demonstrated in him.

*Experiment No. 123.*

*Case No. 60.* L. A., 31 years old. Agent. No diabetes in the family. Always been healthy before. Rather nervous and psychically depressed in the last 6 months. Latterly, has also been troubled by palpitation. On examination he reported that he "had possibly been rather thirsty during the last month," and when he had drunk water in the evening he was obliged to urinate during the night.

On physical examination 27. XII. 22., nothing abnormal was detected. The urine gave strongly positive qualitative sugar reactions (Benedict and Almén). He had eaten 2 sandwiches about 3 hours previously.

28 XII. 22. 9 a. m.: 50 gm. glucose and 200 cc. water.

	Urine cc.	Benedict	Blood sugar
9 a. m.		—	89
9.15 "			100
9.30 "			167
9.45 "			191
10 "	25	+	158
10.15 "		1 per cent.	129
10.30 "		polarimeter	98
10.45 "			91
11 "	20	0.5 per cent.	78

As will be seen from the table, the fasting blood sugar was normal in the morning, and 1½ hours after 50 gm. glucose dissolved in 200 cc. water had been taken the blood sugar curve had again fallen below 110. The maximum blood sugar concentration was within the normal limits. As he could not furnish samples of urine more frequently than every hour it was impossible to determine the position of the threshold, but since the sugar concentration in the first hour's urine was 1.1%, and in the second hour's, 0.5% (polarimeter) in spite of the normal blood sugar rise, the threshold must have been lower than normal.

*Case No. 61.* 30-year-old bookkeeper. No diabetes in the family. Has never before been ill. In November, 1922, his glycosuria was discovered. He has had no thirst and no polyuria.

*Experiment No. 124.*

December 19th, 1922. 8 a. m.: 50 gm. glucose and 200 cc. water.

	Urine cc.	Sugar in urine	Blood sugar
8 a. m.		—	101
8.15 "	10	—	160
8.30 "	9	+	185
8.45 "	16	3%	211
9 "	18	4%	202
9.15 "	15	3.4%	142
9.30 "	8	+	101
9.45 "	6	+	71
10 "	6	—	67

*Experiment No. 125.*

December 20th, 1922. 8 a. m.: 50 gm. glucose and 200 cc. water.

	Urine cc.	Sugar in urine	Blood sugar
8 a. m.		—	123
8.15 "	1	—	138
8.30 "	4	+	215
8.45 "	9	+++	282
9 "	12	+++	258
9.15 "	20	5%	228
9.30 "	8	+++	156
9.45 "	5	+++	119
10 "	5	+	103
10.15 "	5	—	91

In this case two investigations were undertaken with 50 gm. glucose dissolved in 200 cc. water. In the first experiment, No. 124, the fasting blood sugar in the morning was normal and in 1½ hours it was again less than 110. The peak of the blood sugar curve was likewise within the normal limits.

In experiment No. 125, the fasting blood sugar in the morning was 123, but 2 hours after the experiment was started the blood sugar curve had fallen below 110. On the other hand, the blood sugar curve in this experiment was very high, rising to 282. He stated that late in the evening, before the last experiment, he had drunk chocolate and eaten a good many sweet cakes.

It will be noted that in both experiments a considerable degree of glycosuria developed.

The renal threshold was:

Experiment No.	—Blood sugar curve— Rising	Decreasing
124 :	Between 160 and 185	101 and 71
125 :	" 138 " 215	119 " 103



In this case there was a big difference in the position of the threshold during the rising and falling parts of the blood sugar curve. In the rising portion the threshold was at about the usual level—so far as can be judged from the experiments—but in the falling portion it was very low. In the first experiment the glycosuria was dependent principally on the low threshold. In the second experiment the large blood sugar rise also had a great effect in the production of the glycosuria. In this case there again seemed to be a difference in the two experiments on the situation of the threshold in the falling part of the curve, for in experiment 124 there was glycosuria when the blood sugar fell from 101 to 71, but in experiment 125 there was none at blood sugar values between 103 and 91.

In the last case it was hard to decide whether it was an early case of diabetes or an innocent case of glycosuria. There had been no definite subjective symptoms pointing to diabetes and the first experiment only showed a low threshold during the curve's falling portion. But the second experiment showed the presence of an increased fasting blood sugar in the morning and a marked blood sugar rise.

*Case No. 62.* T., 40 years of age. Agent. No diabetes in the family. In 1911, was overworked and nervous; was granted 3 months' sick leave. Otherwise, never had any important illness. In 1919, when he applied for life insurance, glycosuria was discovered. He was accepted, however, without increased premium. Since then he has had his urine tested for sugar at intervals, which was always present, the amount varying between 0.2 and 0.7%. At first he dieted himself, but of late he has eaten all kinds of food except sugar. He never had symptoms of polyuria, thirst, hunger, etc. In 1920, he was on a strict diabetic diet for 3 days, that is to say he ate no carbohydrate, and on the last day he only took soup, but the glycosuria was almost unchanged as the urine contained 0.2% sugar at the end of the 3 days.

I examined him on 29. IV., 3. V., and 6. V. 1921.

#### *Experiment No. 126.*

*April 29th, 1921.* Morning urine (fasting): 0.2% sugar estimated by polarimeter.

Blood sugar at same time: 93 mg.

Then received 20 gm. glucose + 200 cc. water.

Blood sugar 15 minutes later : 126

“ “ 30 “ “ : 151

“ “ 1½ hours “ : 98

Urine 1 hour “ : 2.5% sugar

*May 3rd, 1921.* Has been for 3 days on a diet of 300 gm. bread + 200 gm. potatoes and any additional food he wished.

24 hours' urine: 0.6% sugar.

*May 6th, 1921.* Has been for 3 days on a strictly carbohydrate-free diet, which was also rather restricted.

24 hours' urine: 0.3%.

Morning urine (fasting): 0.1%.

Blood sugar at same time, 97 mg.

*Experiment No. 127.*

He then received 50 gm. glucose + 200 cc. water.

Blood sugar	15 minutes later	:	133	
"	" 30 "	:	182	
"	" 45 "	:	211	
"	" 1 hour "	:	186	Urine 1st hour : 2.8% sugar
"	" 1¼ "	:	165	" 2nd " : 3.2% "
"	" 1½ "	:	140	9 hours later the urine con-
"	" 1¾ "	:	121	tained 0.6% sugar
"	" 2 "	:	100	

Experiment No. 127 showed a rather high blood sugar rise, but not higher than was previously found in these experiments in perfectly healthy individuals. This case had glycosuria constantly, even on a completely carbohydrate-free diet. The cause of this was a very low threshold which was below 93. When the sugar concentration in the 24 hours' urine after 3 days without carbohydrate is compared with the concentration after 3 days on a diet rich in carbohydrate, the difference is not great, being 0.3 and 0.6%, respectively, but if the urine was collected at shorter intervals after the administration of glucose, the glycosuria proved to be greatly dependent upon the introduction of carbohydrate.

*Case No. 63.* H. T. 39 years of age. Doctor. Brother of the preceding patient. Never had any important illness. For a long time he has observed that after a meal rich in carbohydrate, Almén's reaction in the urine was positive. Otherwise no symptoms of diabetes.

*Experiment No. 128.*

August 2, 1922. 9 a. m.: 50 gm. glucose and 200 cc. water.

	Urine		Benedict	Blood sugar
	cc.			
8 to 9 a. m.	28		—	9 a. m. : 100
9.15 "	1			9.15 " : 112
9.30 "	0			9.30 " : 153
9.45 "	2		+	9.45 " : 174
10 "	25		+++	10 " : 160
10.15 "	5		+++	10.15 " : 133
10.30 "	8		+++	10.30 " : 105
10.45 "	6		+	10.45 " : 98
11 "	6		+	11 " : 95

*Experiment No. 129.*

August 3rd, 1922. 9 a. m.: 50 gm. bread and 200 cc. water.

	Urine cc.	Benedict	Blood sugar
8 to 9 a. m.	26	—	9 a. m. : 90
9.15 "	5	—	9.15 " : 95
9.30 "	5	—	9.30 " : 104
9.45 "	7.5	—	9.45 " : 113
10 "	5	—	10 " : 114
10.15 "	5	—	10.15 " : 98
10.30 "	7.5	—	10.30 " : 90
10.45 "	7	—	10.45 " : 87
11 "	5	—	11 " : 85

*Experiment No. 130*

August 4th, 1922. 9 a. m.: 100 gm. bread and 200 cc. water.

	Urine cc.	Benedict	Blood sugar
8 to 9 a. m. :	28	—	9 a. m. : 90
9.15 "	0		90
9.30 "	0		110
9.45 "	0		121
10 "	15	—	133
10.15 "	0		140
10.30 "	10	+	120
10.45 "	0		110
11 "	15	—	103

In experiment No. 128, the threshold on the rising part of the blood sugar curve was between 112 and 174, while it on the decreasing part was between 105 and 98.

It is evident from the experiments that the cyclic glycosuria in this person is due to a low renal threshold, which causes elimination of sugar after carbohydrates.

*Case No. 64.* A manager, 34 years old. His father has had diabetes for several years.

In 1915, he had a boil on one finger which was the cause of his urine being tested and sugar found. Since then he has dieted himself to some extent. Every day he has consumed some oatmeal porridge, fruit, and up to 100 gm. bread, green vegetables ad lib., and otherwise carbohydrate-free food. The 24 hours' sample of urine has practically constantly contained 0.3 to 0.7% sugar. He has never suffered from thirst and never had polyuria or polyphagia.

He was rather small and perhaps somewhat thin, otherwise there was nothing to note.



*Experiment No. 131.*

February 17th, 1923. 9 a. m.: 50 gm. glucose and 200 cc. water.

	Blood sugar	Urine cc.	Benedict
9 a. m.	: 107		—
9.15 "	: 135	12	—
9.30 "	: 183	10	++
9.45 "	: 232	11	+++
10 "	: 197	21	+++
10.15 "	: 174	14	+++
10.30 "	: 149	13	+++
10.45 "	: 114	11	++
11 "	: 100	6	+

The blood sugar before the experiment and also 2 hours after was less than 110, but in both instances it was higher than is generally found in healthy persons. The maximum of the blood sugar rise, 232, was higher than I have found in normal individuals. The renal threshold's level was very different in the rising and falling parts of the curve; in the rising portion it did not deviate from the normal to an appreciable extent, but in the falling portion it was very low. The glycosuria was partly due to the low threshold and partly to the high blood sugar rise.

Of the 8 cases discussed under the heading "transition cases," 2 were discovered in the experiments undertaken to study the blood sugar and urine sugar in normal persons after taking carbohydrates. These experiments were made on 25 individuals, all of whom were apparently quite healthy, but among these 25 the experiments brought to light two—both medical students—who had low thresholds and consequently a large sugar excretion after taking 50 gm. glucose. A third student, case No. 18 (see experiments 79 and 80) also possessed a lower threshold than is commonly encountered in healthy people, but in his case the sugar concentration in the urine was relatively small and he is therefore not included among the transition cases.

The previous 6 examined had already noticed their glycosuria before these experiments were carried out.

Six of the eight transition cases only showed signs of a low renal threshold, the situation of which varied greatly from one individual to another. In No. 62, the threshold was so low that glycosuria was constantly present. In the other 5 it was higher, and glycosuria appeared only after taking carbohydrate. One of the cases, No. 61, also exhibited two other abnormalities, namely, increased fasting blood sugar on the one day and a remarkably

high blood sugar curve on the 2nd day. In the last case, No. 64, a combination of a low threshold and a high blood sugar rise was met with.

#### IV. *Investigations in Diabetics.*

The sugar excretion in the 24 hours' urine and after taking small amounts of glucose and various foods was determined. In addition, some investigations into the renal threshold under different conditions were made.

##### *Experiment No. 132.*

##### *24 hours' sugar excretion.*

*Case No. 5.* 9-year-old girl, who had had diabetes since 1918.

Day Aug.	Urine cc.	Bene- dict	Total sugar		Fermented		Calories	Carbohy- drate
			pro mil.	mg.	pro mil.	mg.		gm.
15	1200	—	0.32	384	0.12	144	600	30
16	1450	—	0.39	566	0.17	247	700	30
17	1400	—	0.29	406	0.16	224	.....	....
18	1300	—	0.38	494	0.24	312	750	....
19	900	—	0.37	333	0.19	171	.....	....
20	1000	—	0.65	650	0.44	312	800	47
21	900	+	2.43	2187	1.86	1674	800	57
22	850	—	0.60	510	0.30	255	45	9
23	700	—	0.26	182	0.07	49	45	9
24	1000	—	0.22	220	0.0	0	300	22
25	1100	—	0.19	209	0.04	44	.....	....
26	600	—	0.23	138	0.05	30	400	....
27	1100	—	0.17	187	0.06	66	600	....
28	1700	—	0.20	340	0.08	136	700	....
29	1000	—	0.30	300	0.14	140	.....	....

For the first 5 days the urine was examined, the patient received 600 to 750 calories and about 30 gm. carbohydrate per day, during which time the total sugar varied between 333 and 566 mg., while the fermentable sugar varied between 144 and 312 mg. On August 20, the number of calories was increased to 800 and the amount of carbohydrate to 47 gm. Even by the 21st the amount of fermentable sugar was considerably increased and simultaneously Benedict's sugar reaction became positive. On the succeeding days the patient was put on a very low diet, which caused the total sugar and particularly the fermentable sugar to fall greatly. On the 24th, no fermentation of excreted sugar was obtained (but fermentation of glucose added to the urine as a control, took place). The number of calories was then augmented for a few days, and the sugar excretion rose to roughly the same amount as before the glycosuria appeared.

*Case No. 6.* Even on the first day on which the urine sugar was estimated (Aug. 18), the excretion of fermentable sugar was about five times greater than is usually found in healthy persons, but on account of the great diuresis the sugar concentration was too small to demonstrate the qualitative reactions. Next day, the 19th, the sugar excretion was further increased and now reached so high a concentration that the qualitative sugar reaction was positive. During the subsequent period on a low diet the excretion fell rather suddenly and sharply, and fluctuated between August 21 and 30 from 570 to 800 mg. total sugar, and from 209 to 429 mg. fermentable sugar. On August 31, the number of calories was increased to 1900, and at the same time the fermentable sugar rose to more than thrice the amount, but Benedict's reaction was negative, the large diuresis keeping the concentration down to 0.65 pro mille.

*Experiment No. 133.*

*Case No. 6. Woman, 62 years old.*

Day	Urine	Bene-	Total sugar		Fermented		Calories	Carbohy-
Aug.	cc	dict	pro mil.	mg.	pro mil.	mg.		drate
18	2200	—	0.95	2090	0.76	1670	1600	20
19	1800	+	2.10	3780	1.97	3550	.....	....
20	2000	—	0.63	1260	0.50	1000	700	5
21	2000	—	0.31	620	0.13	260	.....	....
22	1900	—	0.32	608	0.13	247	800	10
23	1950	—	0.41	800	0.22	429	1000	20
24	2000	—	0.38	760	0.21	420	1200	23
25	1900	—	0.30	570	0.11	209	1400	....
26	1800	—	0.37	666	0.22	396	.....	....
27	2000	—	0.32	640	0.16	320	1600	....
28	2200	—	0.33	726	0.15	330	.....	....
29	1900	—	0.35	665	0.19	361	1700	....
30	2000	—	0.35	700	0.20	400	.....	....
31	2200	—	0.90	1980	0.65	1430	1900	....



*Experiment No. 134.**Case No. 7. 33-year-old man.*

Day Aug.	Urine cc	Bene- dict	Total sugar		Fermented		Calories	Carbohy- drate
			pro mil.	mg.	pro mil.	mg.		gm.
21	1300	—	0.53	636	0.23	276	1600	15
22	1700	—	0.43	731	0.15	255	.....	....
23	1050	—	0.89	935	0.52	546	.....	25
24	1350	+	2.33	3146	1.93	2606	.....	....
25	1900	—	0.47	893	0.33	627	1300	....
26	950	—	0.91	865	0.53	504	.....	....
27	1500	—	0.50	750	0.23	345	.....	....
28	1750	—	0.93	1628	0.84	1470	1400	28
29	1600	—	1.20	1920	0.90	1440	.....	....
30	1700	+	1.50	2550	1.20	2040	.....	....
31	1700	+	2.02	3434	1.79	3043	.....	....
Sept.								
1	1800	+	1.20	2160	1.04	1872	500	20
2	1900	—	0.50	950	0.30	570	320	18
3	1700	—	0.32	544	0.17	131	800	24
4	lost						1075	....
5	3500	+	0.85	2975	0.68	2380	.....	....
6	lost						1400	....
7	1700	+	1.43	2431	1.20	2040	1700	24
8	lost							
9	1100	—	1.30	1430	0.92	1012	1000	20
10	1300	—	1.35	1755	0.96	1248	.....	....
11	1800	—	0.87	1566	0.60	1080	.....	....

*Experiment No. 135.**Case No. 8. 41-year-old man.*

Day Aug.	Urine cc	Bene- dict	Total sugar		Fermented		Calories	Carbohy- drate
			pro mil.	mg.	pro mil.	mg.		gm.
21	2300	+	3.40	7820	3.18	7310	400	18
22	2500	—	0.23	575	0.10	250	.....	....
23	3700	—	0.24	888	0.09	333	60	12
24	3100	—	0.27	837	0.08	248	150	15
25	2800	—	0.30	840	0.16	448	.....	....
26	2700	—	0.30	810	0.17	459	610	....
27	3000	—	0.26	780	0.15	450	995	....
28	2800	—	0.38	1064	0.23	644	1300	25
29	2800	—	0.40	1120	0.25	700	1400	30
30	lost							
31	2900	—	0.45	1305	0.30	870	1600	....

*Experiment No. 136.**Case No. 9. Woman, 56 years old.*

Day Aug.	Urine cc	Bene- dict	Total sugar		Fermented		Calories	Carbohy- drate
			pro mil.	mg.	pro mil.	mg.		gm.
23	800	+++	3.33	2664	2.93	2344	0	0
24	800	—	0.67	536	0.27	216	45	9
25	1600	—	0.37	592	0.18	228	.....	....
26	1800	—	0.30	540	0.11	198	.....	....
27	1900	—	0.37	703	0.16	304	105	20
28	1600	—	0.37	592	0.16	256	205	....
29	1700	—	0.36	612	0.17	289	300	36
30	lost						.....	....
31	2400	—	0.34	816	0.13	312	430	....
Sept.								
1	1950	—	0.20	390	0.07	117	530	41
2	2000	—	0.20	400	0.08	160	800	45
3	2200	—	0.17	374	0.07	154	1300	....
4	lost							
5	lost							
6	"	"						
7	2000	+	0.27	540	0.10	200	1800	....
8	2050	+	0.24	492	0.10	205	.....	....

Experiments 134-136, like the preceding two, showed that when the number of calories and the amount of carbohydrate are kept below a certain maximum for each individual, the excretion of sugar is about the same as in healthy persons. Let the calories or carbohydrate be increased above this maximum and the fermentable sugar suddenly rises greatly, while Benedict's qualitative sugar reaction becomes positive. The marked increase in the fermentable sugar was often detected one or more days before the qualitative sugar reaction was positive, and it often persisted, on changing to a restricted diet, for several days, although the qualitative reaction was negative, and then suddenly fell to between 200 and 500 mg. per diem. The increased sugar excretion involved almost entirely the fermentable sugar, as the non-fermentable part of the total sugar was about the same whether the excretion of fermentable sugar was large or small.

In the 9-year-old girl, the sugar excretion fell when she was on a low diet, but in none of the other patients was there any diminished sugar excretion while their diet was restricted.

*The influence of glucose and of some foods on urine sugar and on blood sugar in diabetics.*

*Experiment No. 137.*

*Case No. 32.*

February 15th, 1922. 8 a. m.: 100 gm. bacon, 300 gm. cabbage and 50 cc. broth.

		Urine cc.	Bene- dict	Total sugar pro mil.	sugar mg.	Fermented pro mil.	mg.	Blood sugar
7 to 8	a. m.	: 31	—	0.90	28	0.40	12	8 a. m. : 156
	8.30 "	: 19	—	1.19	31	0.65	17	8.30 " : 157
	9 "	: 7	+					9 " : 183
	9.30 "	: 13	++	1.95	59	1.40	42	9.30 " : 180
	10 "	: 17	++					10 " : 175
	10.30 "	: 13	++	1.81	60	1.20	40	10.30 " : 170
	11 "	: 20	+					11 " : 169

*Experiment No. 138.*

*Case No. 32.*

February 16th. 8 a. m.: 100 gm. meat ball, 300 gm. cabbage, 50 cc. broth.

		Urine cc.	Bene- dict	Total sugar pro mil.	sugar mg.	Fermented pro mil.	mg.	Blood sugar
7 to 8	a. m.	: 24	—	1.17	28	0.52	12	8 a. m. : 151
	8.30 "	: 15	+	1.60	62	1.06	41	8.30 " : 184
	9 "	: 24	+					9 " : 197
	9.30 "	: 23	++	2.84	143	2.19	112	9.30 " : 188
	10 "	: 28	++					10 " : 184
	10.30 "	: 35	++	1.10	75	0.70	45	10.30 " : 176
	11 "	: 33	+					11 " : 171

In both experiments the blood sugar as well as the fermentable sugar increased, and Benedict's reaction became positive. The increase was, however, greatest after meat.

*Experiment No. 139.*

*Case No. 47.*

8 a. m.: Mixed meal, containing cabbage, one banana, 3 crackers, etc.

		Urine cc.	Bene- dict	Total sugar pro mil.	sugar mg.	Fermented pro mil.	mg.	Blood sugar
7 to 8	a. m.	- 50	—	0.45	23	0.18	9	8 a. m. : 118
								8.30 " : 150
8 "	9 "	39	—	0.70	20	0.30	12	9 " : 190
								9.30 " : 190
9 "	10 "	44	+	1.16	51	0.56	25	10 " : 173
								10.30 " : 148
10 "	11 "	55	—	0.68	38	0.32	18	11 " : 135



*Experiment No. 140.**Case No. 47.*

8 a. m.: 100 gm. bacon and 200 cc. coffee.

		Urine		Total sugar			
		cc.	Benedict	pro mil.	mg.	Blood sugar	
7 to 8 a. m.	:	35	—	0.91	32	8 a. m.	: 96
						8.30 "	: 98
8 " 9 "	:	21	—	1.44	30	9 "	: 100
						9.30 "	: 98
9 " 10 "	:	21	—	1.62	34	10 "	: 98
						10.30 "	: 96
10 " 11 "	:	28	—	1.20	34	11 "	: 97

*Experiment No. 141.**Case No. 47.*

8 a. m.: 300 gm. meat and 200 cc. water.

		Urine		Total sugar			
		cc.	Benedict	pro mil.	mg.	Blood sugar	
7 to 8 a. m.		54	—	0.35	38	8 a. m.	: 102
						8.30 "	: 102
8 " 9 "		50	—	0.70	35	9 "	: 106
						9.30 "	: 118
9 " 10 "		55	—	0.72	40	10 "	: 118
						10.30 "	: 106
10 " 11 "		43	—	0.80	34	11 "	: 106

Even after 300 gm. meat there was no definite rise in the total blood sugar and, therefore, fermentation was not carried out. The blood sugar showed a distinct but not large rise after the meat meal.

*Experiment No. 142.**Case No. 47*

8 a. m.: 500 gm. cabbage, boiled once, and 200 cc. water.

		Urine	Bene-	Total sugar		Fermented			
		cc.	dict	pro mil.	mg.	pro mil.	mg.	Blood sugar	
7 to 8 a. m.	:	33	—	0.81	26	0.31	10	8 a. m.	: 104
								8.30 "	: 119
8 " 9 "	:	20	—	1.36	27	0.52	10	9 "	: 124
								9.30 "	: 116
9 " 10 "	:	30	—	0.96	29	0.42	13	10 "	: 110
								10.30 "	: 104
10 " 11 "	:	34	—	0.72	24	0.34	12	11 "	: 104

500 gm. cabbage did not produce a definite increase in the sugar excretion, but there was a rise in the blood sugar.

*Case No. 45.* P. R. S., sailor, 43 years old, weight 72 kg. No diabetes in the family. Had never had any important illness before, but had been nervous for many years. In 1920, his doctor discovered he had glycosuria. In February, 1922, he was again examined by the doctor, who found glycosuria still present. He had never dieted himself, in fact he had taken much sugar daily. At times he had suffered greatly from thirst, but he had not become thinner.

On admission to the Medical Department A, on July 21, 1922, he did not feel ill, and as there was not more than 0.4% sugar in the urine, despite his having daily indulged in large quantities of carbohydrate, a tolerance test was first done with 50 gm. bread.

*Experiment No. 143.*

July 22nd, 1922. 9 a. m.: 50 gm. bread and 200 cc. water.

	Urine cc.	Benedict	Blood sugar
9 a. m. :		—	112
9.30 " :	11	+	155
10 " :	18	++	205
10.30 " :	22	+++	169
11 " :	17	+++	155

The blood sugar rise was high and of long duration, typical in diabetics.

*Experiment No. 144.*

August 22nd, 1922. 8 a. m.: 6 gm. glucose and 200 cc. water.

	Urine cc.	Bene- dict	Total sugar pro mil. mg.	Fermented pro mil. mg.	Blood sugar
7 to 8 a. m. :	29	—	1.17 34	0.50 15	8 a. m. : 109
8.15 " :	6	+	2.44 81	2.08 69	8.15 " : 129
8.30 " :	8	++			8.30 " : 131
8.45 " :	10.5	+			9 " : 109
9 " :	8.5	++			8.45 " : 126
9.15 " :	8	+	1.38 39	1.05 29	9.15 " : 104
9.30 " :	6.5	—			9.30 " : 100
9.45 " :	7.5	—			9.45 " : 96
10 " :	5.5	—			10 " : 92

*Experiment No. 145.*

August 23. 8 a. m.: 6 gm. glucose and 200 cc. water.

		Urine	Bene-	Total sugar	Fermented		Blood sugar	
		cc.	dict	pro mil.	mg.	pro mil.	mg.	
7 to 8	a. m.	: 23	—	0.81	19	0.40	9	8 a. m. : 116
8.15	"	: 16	—					8.15 " : 123
8.30	"	: 10	trace					8.30 " : 127
8.45	"	: 17	trace	0.64	51	0.37	29	8.45 " : 125
9	"	: 36	—					9 " : 118
9.15	"	: 45	—					9.15 " : 100
9.30	"	: 25	—					9.30 " : 95
9.45	"	: 21	—	0.30	32	0.10	11	9.45 " : 96
10	"	: 15	—					10 " : 94

The last two experiments were quite alike, but in the first the blood sugar rise was greater than in the second, and in conformity with this the fermentable sugar in the first experiment was considerably increased, while in the second the urine sugar was less increased. The renal threshold is, as will be seen, between 120 and 130.

*Experiment No. 146.*

August 21st, 1922. 8 a. m.: 300 gm. meat and 200 cc. water.

		Urine	Bene-	Total sugar	Fermented		Blood sugar	
		cc.	dict	pro mil.	mg.	pro mil.	mg.	
7 to 8	a. m.	: 35	—	1.0	35	0.50	18	8 a. m. : 101
8.30	"	: 12	+					8.30 " : 105
9	"	: 22	++	3.89	132	3.02	103	9 " : 117
9.30	"	: 20	++					9.30 " : 115
10	"	: 17	++	5.54	205	3.44	127	10 " : 114
10.30	"	: 15	++					10.30 " : 114
11	"	: 17	++	2.55	82	1.40	45	11 " : 113

In the urines from 8 to 11 a. m., the phenylhydrazin reaction was positive.

*Experiment No. 147.*

August 30th. 8 a. m.: 300 gm. of meat and 200 cc. water.

		Urine	Total sugar		Fermented		Blood sugar	
		cc.	Benedict	pro mil.	mg.	pro mil.	mg.	
7 to 8	a. m.	51	—	0.64	33	0.28	14	8 a. m. : 107
								8.15 " : 111
8.30	"	7	—					8.30 " : 113
				2.0	29	1.0	15	8.45 " : 113
9	"	7.5	—					9 " : 113
								9.15 " : 114
9.30	"	17.5	trace					9.30 " : 113
				2.45	99	0.90	37	9.45 " : 113
10	"	23	trace					10 " : 111
10.30	"	24	trace					10.30 " : 110
				1.95	82	0.80	34	
11	"	18	trace					11 " : 109



These two experiments, like the two preceding ones, were absolutely alike. They both showed a slight increase in the blood sugar, rather greater in the first than in the second experiment. In the first experiment, there was a considerable increase in the fermentable sugar, and Benedict's sugar reaction and the phenylhydrazin test were positive. In the second, there was a definite but not very great increase in the fermentable sugar and Benedict's sugar reaction gave a "trace" in several urine samples.

When we compare the two experiments where 6 gm. glucose was given with the two where 300 gm. meat was given, it will be noticed that the increase in the sugar excretion in the last two experiments comprises both fermentable and "non-fermentable sugar," but in the two experiments where glucose was given, only the fermentable sugar was increased. Moreover, in the experiments with glucose the sugar excretion was, as a rule, less than in those relating to meat, in spite of the fact that the blood sugar rise was greater in the former, which would indicate that the renal threshold was lower with meat meals than after taking glucose.

*Case No. 43.* B. O., Sailor, 22 years old. No diabetes in the family. At 10 years of age he had "inflammation of the brain." Two months ago he began to suffer from great thirst, and on admission to the Medical Department A on July 20, 1922, he had 5.5% sugar in the urine.

*Experiment No. 148.*

August 1st. 8 a. m.: 6 gm. glucose and 200 cc. water.

		Urine	Bene-	Total sugar		Fermented		Blood sugar
		cc.	dict	pro mil.	mg.	pro mil.	mg.	
7 to 8	a. m.	70	—	0.40	28	0.20	14	8 a. m. : 133
8.15	"	8	—	0.55	25	0.30	14	8.15 " : 149
8.30	"	10	—					8.30 " : 174
8.45	"	14	—	0.43	25	0.24	14	8.45 " : 163
9	"	13	—					9 " : 153
9.15	"	15	—					9.15 " : 151
9.30	"	13	—					9.30 " : 150
9.45	"	14	—	0.43	25	0.24	14	9.45 " : 142
10	"	16	—					10 " : 136

There was an increase of the blood sugar concentration, which did not influence the urine sugar.

*Experiment No. 149.*

August 14th. 8 a. m.: 15 gm. sodium bicarb. and 200 cc. water.

	Urine cc.	Benedict	Total sugar pro mil.	mg.	Blood sugar
7 to 8 a. m.	50	—	0.50	25	8 a. m. : 112
					8.15 " : 112
8 " 9 "	37	—	0.60	22	8.30 " : 113
					8.45 " : 112
9 " 10 "	48	—	0.55	26	9 " : 113
					9.15 " : 112
10 " 11 "	92	—	0.30	28	9.30 " : 112
					9.45 " : 113
					10 " : 112
					10.30 " : 112
					11 " : 112

Sodium bicarbonate did not cause any change in the sugar in blood or urine.

*Experiment No. 150.*

August 15th. 8 a. m.: 6 gm. glucose, 15 gm. sodium bicarb. and 200 cc. water.

	Urine cc.	Bene- dict	Total sugar pro mil.	mg.	Fermented pro mil.	mg.	Blood sugar
7 to 8 a. m.	108	—	0.24	26	0.10	11	8 a. m. : 112
8.15 "	10	—	0.82	24	0.33	10	8.15 " : 131
8.30 "	6	—					8.30 " : 140
8.45 "	6	—					8.45 " : 144
9 "	7	—					9 " : 150
9.15 "	12	—	0.33	24	0.10	8	9.15 " : 144
9.30 "	17.5	—					9.30 " : 133
9.45 "	25	—					9.45 " : 129
10 "	23	—					10 " : 124

Sodium bicarbonate did not influence the blood sugar rise after 6 gm. of glucose, since the increase of blood sugar concentration in this experiment was equal to the increase in experiment No. 143.

*Experiment No. 151.*

August 16th. 8 a. m.: 2 cc. dilute hydrochloric acid, 6 gm. glucose and 200 cc. water.

		Urine	Benedict	Total sugar		Blood sugar
		cc.		pro mil.	mg.	
7 to 8	a. m.	86	—	0.27	23	119
8.15	"	14	—	0.48	19	128
8.30	"	7	—			142
8.45	"	15	—			145
9	"	14	—			135
9.15	"	18	—	0.40	23	131
9.30	"	14	—			128
9.45	"	17	—			124
10	"	8.5	—			124

Nor had hydrochloric acid any influence on the blood sugar rise or on the urine sugar.

*Experiment No. 152.*

August 17th. 8 a. m.: 300 gm. meat and 200 cc. water.

		Urine	Bene-	Total sugar		Fermented	Blood sugar	
		cc.	dict	pro mil.	mg.	pro mil.	mg.	
7 to 8	a. m.	60	—	0.50	30	0.20	12	8 a. m. : 116
8	" 9 "	65	—	0.40	26	0.20	13	9 " : 131
								9.15 " : 138
9	" 10 "	60	—	0.88	53	0.40	24	9.30 " : 138
								9.45 " : 137
10	" 11 "	50	—	0.96	48	0.45	23	10 " : 135
								10.15 " : 133
								10.30 " : 130
								10.45 " : 128
								11 " : 124

After 300 gm. of meat the sugar in blood and in urine was increased.

*Experiment No. 153.*

August 19th. 7 a. m.: 300 gm. meat, 200 cc. water.

8 a. m.: 6 gm. glucose and 200 cc. water.

		Urine	Bene-	Total sugar		Fermented	Blood sugar	
		cc.	dict	pro mil.	mg.	pro mil.	mg.	
6 to 7	a. m.	: 165	—	0.17	28	0.05	8	
7 to 8	"	: 170	—	0.20	34	0.05	9	8 a. m. : 128
8.15	"	: 38	trace	1.10	175	0.80	127	8.15 " : 176
8.30	"	: 45	trace					8.30 " : 169
8.45	"	: 47	+					8.45 " : 167
9	"	: 29	+					9 " : 163
9.15	"	: 39	+	0.50	80	0.28	45	9.15 " : 158
9.30	"	: 33	—					9.30 " : 150
9.45	"	: 56	—					9.45 " : 142
10	"	: 32	—					10 " : 135



6 gm. of glucose one hour after 300 gm. of meat, at the time of digestion, gave the same blood sugar rise as after glucose only (see experiment No. 148). The digestion, or "the external function of the pancreas," does not influence the blood sugar rise in diabetics.

The urine sugars, however, were different in the two experiments.

While there was no change in the amount of fermentable urine sugar in the experiment with glucose only, a considerable increase in the sugar excretion took place when glucose was given during the digestion of meat.

The results of the experiments on the last two persons agree well with one another, both showing a lower renal threshold during the digestion of meat than after taking glucose.

#### V. *Investigations into the Occurrence of Glucose in Normal Urine.*

Experience shows that Almen's and Benedict's reactions are usually negative in the urine of healthy persons, and that a strong positive reaction is due to glycosuria. Nevertheless, these reactions are not infrequently weakly positive in the concentrated urine of patients displaying no symptoms of diabetes, as the following table shows.

##### *Experiment No. 154.*

Urine No.	Spec. gravity	Almén's reaction	Benedict's reaction
1	1025	+	+
2	1025	trace	+
3	1023	trace	+
4	1031	+	+
5	1025	+	+
6	1030	+	+
7	1025	+	trace
8	1028	trace	+

The urine came from patients who were febrile, or were passing less water in consequence of cardiac stasis, or had taken little to drink. None of the samples contained albumin.

A series of experiments has proved to me that when the urine from normal persons is evaporated to one-tenth of its volume, it will usually give a positive Benedict reaction and not uncom-

monly a positive Almen test also. This, in conjunction with the fact that both tests are reduction processes reacting with many substances (pentoses, glycuronic acid and many others), which may be present in normal urine, makes it obvious that neither of these reactions is adapted for solving the problem whether glucose is a normal constituent of the urine.

The case is different with the *phenylhydrazin reaction*, which, as is known, is based on the formation of definite crystal forms. This reaction was first described by Emil Fischer,<sup>51</sup> who showed that when different kinds of sugar in watery solution were boiled with phenylhydrazin chloride and sodium acetate, crystals of characteristic appearance and melting point separated out on cooling. A couple of years later von Jaksch<sup>52</sup> recommended the phenylhydrazin test for demonstrating sugar in the urine and proved that 1 pro mille sugar could be detected with great certainty.

This reaction has never gained wide clinical application, no doubt chiefly because, in comparison with the reduction reactions, it is laborious, and perhaps partly also because there has been a difference of opinion as to how a positive reaction shall be construed clinically. In consequence of this, opinion has also been divided as to whether the phenylhydrazin reaction can be positive in the urine of healthy persons. Thus Moritz<sup>53</sup> and Roos<sup>54</sup> always found a positive reaction in normal urine, while Hirschl<sup>55</sup> and later Geelmuyden<sup>56</sup> never found the reaction positive in the urine of healthy persons. On the basis of his experiments, Geelmuyden asserted that the sugar occurring in normal urine, which he termed "physiological sugar," could not be glucose. This conclusion is, however, not tenable, since the limit of sensitiveness of the phenylhydrazin reaction in the form employed by Geelmuyden is hardly lower than 0.5 pro mille glucose in urine (see later) and therefore glycosuria of 0.3-0.4 pro mille and less could not be detected.

The phenylhydrazin reaction is not an absolutely specific test for glucose, as fructose, at any rate, gives exactly the same osazone as glucose. Some workers hold that cane sugar (Rosenfeld<sup>57</sup>), glycuronic acid, pentoses, and lactose (Margulies<sup>58</sup>), and galactose (Neuberg<sup>59</sup>) also form the same osazones, but this is denied, for example by Hirschl<sup>55</sup>, who maintains that only fructose gives the same form of crystals as glucose.

Although the phenylhydrazin reaction is therefore not a completely specific test for glucose, it reacts with many fewer substances than the reduction reactions and it is consequently decidedly more suitable for investigating the occurrence of glucose in normal urine.

When Fischer<sup>51</sup> first described the phenylhydrazin reaction, sufficiently pure phenylhydrazin was not on the market and he therefore used the hydrochloride. This salt was also employed by von Jaksch<sup>52</sup> among others, and later by Geelmuyden.<sup>56</sup>

In the first two years of the present experiments on urine sugar the phenylhydrazin reaction was occasionally used when Benedict's reaction was positive (see earlier experiments) in the way described by von Jaksch, Geelmuyden, and others. By this method I have found that the reaction is not particularly sensitive, as 0.5-1 pro mille glucose must be added to normal urine, according to the concentration of the urine, to make it positive.

When pure phenylhydrazin was put on the market, Fischer<sup>60</sup> began to use it and several modifications were elaborated, all of which possessed greater sensitiveness than Fischer's original method with phenylhydrazin hydrochloride, the reason being that hydrochloric acid inhibited the sensitiveness. Of the methods advocated using pure phenylhydrazin I have tried Coppolina's<sup>61</sup> and Neumann's<sup>62</sup>. I prefer the latter, which I have somewhat modified as follows:

To about 4 cc. urine in a test tube is added  $1\frac{1}{2}$  to 2 cc. of a saturated solution of sodium acetate in 50% acetic acid, and 3 drops of phenylhydrazin. This is brought to the boil and the tube is then put in a water bath at 100°C for about an hour. The test tube is allowed to remain in the water bath so as to cool slowly and is not examined microscopically until the next day.\*

The crystals formed in *solutions of glucose in water* were first studied by means of this reaction.

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\* Neumann uses 5 cc. urine + 2 cc. sodium acetate — acetic acid solution + 2 or 3 drops of phenylhydrazin and evaporates it by boiling, to about 3 cc. It is then cooled in running water, boiled again, cooled in the same manner, and then subjected to microscopic examination. A special kind of test tube is employed with a considerable expansion some distance above the bottom, contrived, no doubt, to prevent the liquid boiling over. I tried this method but with indifferent result. Moreover, as I also wished to investigate, concentrated urine which very readily "bumps" and boils over, I preferred only to heat up once, keep in the water bath one hour, and examine with the microscope next day, which in my experience gives the most uniform results.



*Experiment No. 155.*

1. 4 cc. distilled water + 2 cc. sodium acetate — acetic acid solution + 3 drops phenylhydrazin. Macroscopically, no precipitate. Under the microscope a number of quite round, homogeneous, reddish-brown spheres were seen. No crystals.

2. 4 cc. 0.025 pro mille glucose in water. Otherwise as above.

3. 4 cc. 0.05 pro mille glucose in water. Otherwise as above.

The microscopic appearance of the precipitate in these two samples was similar. A number of rather fine, yellow needles were observed, arranged singly and also in plumages, and one irregularly formed sheaf. (See drawing.)

4. 0.075 pro mille glucose solution investigated as above. Typical small sheaves were present and some needles.

5. 0.10 pro mille glucose solution. Microscopically a network of fine needles and a few sheaves.

6. 0.15 pro mille glucose solution. Macroscopically a yellow crystalline precipitate, which on microscopic examination was found to consist of large sheaves.

7. 0.20 pro mille glucose solution. Microscopically the same as in Nos. 5 and 6.

8. 0.30 pro mille glucose solution. Microscopically large sheaves and some curved needles.

From these experiments it will be seen that the glucosazone crystals, which form in watery solution, consist of fine needles, which in low concentrations of glucose lie singly and are comparatively short, but which in greater concentrations are partly collected in bundles often arranged in the form of sheaves. It will be further observed that characteristic crystals can be detected in so low a concentration as 0.025 pro mille.

After having gained some knowledge of the course of the phenylhydrazin reaction in watery solutions, about 100 samples

of urine from healthy individuals and convalescents were examined by the method described. The samples were partly early morning ones and partly after various meals. In all of them different formations were observed which, following Geelmuyden, I can class under the heading "physiological osazones." The main types of these osazones seem to be of two kinds, (1) yellow almost homogeneous spheres; (2) flat, wide, rather irregularly formed needles.

Irregular, round, yellow, radially grooved "cakes" were, however, most often met with. These "cakes" varied greatly in size and the larger they were, the more irregular did they become in the periphery and on the surface, and the radial lines were more distinct. A further development seems to be that these grooved masses acquire outrunners consisting of broad, irregularly-shaped needles. Sometimes these outrunners are arranged fairly regularly round the middle, forming rosettes.

The less developed forms, the yellow spheres and the small "cakes" with slightly marked radial lines, are most often found in the overnight urine, while the more developed forms, the large "cakes" with pronounced radial lines and the large rosettes, are observed in urine after meals.

A number of experiments were next made on the crystal form of the glucosazone in the urine of healthy persons to which was added 0.5-1 pro mille glucose. In urine to which glucose has been added single fine needles are not often found, such as are seen in solutions of glucose in water, the reason of which may be that they are difficult to demonstrate amongst all the detritus present in the urine precipitate. But the typical regularly formed "sheaves" are found and also circular dark-colored spheres which are usually constricted at the middle, forming two quite symmetrical halves that appear to consist of fine, short, compact needles radiating out in all directions, in contrast to the physiological osazones which have the appearance of being flat.

As points in the differential diagnosis between the most developed physiological osazones and the spherical or semi-spherical glucosazones the following may be cited.

*Physiological Osazone*

Coarse, flat, irregularly shaped needles.

Needles more or less irregularly disposed.

Osazone crystals are rather flat.

Osazones are relatively light-colored.

*Glucosazone*

Long, narrow, pointed needles.

Needles always uniformly radially disposed.

Osazone crystals are spherical.

Osazones are dark-colored.

The typical sheaves, which are easy to recognize, are not taken into account here. The reader is referred to Geelmuyden's excellent drawings in the "Norsk Magazin f. Laegevidenskaben," 1915, p. 989, but I am doubtful whether Geelmuyden's drawing "f" is a glucosazone or not, because he considers it is a physiological osazone, and I have never seen this form in the urine of healthy persons, though often in normal urine to which small quantities of glucose had been added. Moreover, in Geelmuyden's plate it will be seen that the osazone "f" can hardly be distinguished from the osazone which is below and to the right, in group "1." The reason Geelmuyden considers "f" to be a physiological osazone, is, no doubt, that in the preparation of his physiological osazones he has used urine from "sugar-free" diabetics, that is to say, urine in which Almen's and Worm Muller's reactions are negative, but which, of course, may have contained small amounts of glucose.

The sensitiveness of the phenylhydrazin reactions in the form described was next investigated in urine to which varying amounts of glucose were added. On the addition of small amounts (0.01-0.05 pro mille) to urine the microscopic appearance of the precipitate in the phenylhydrazin reaction was the same as without the sugar. With rather larger additions of glucose, about 0.10 pro mille, the physiological osazones disappeared and were replaced by glucosazones. The transition was usually very sudden and only in a few cases, with a rather lower glucose addition, a preliminary stage consisting of somewhat irregularly shaped osazones appeared to be formed, which were difficult to classify. It was curious to observe how suddenly the physiological osazones gave place to the glucosazones. Only in some cases were a few physiological osazones found in company with the



glucosazones when only a little sugar had been added to the urine. If the sugar added reached a certain amount the physiological osazones completely disappeared.

These investigations have shown that typical glucosazones are formed in the majority of urines when 0.10 pro mille glucose is added to them; in some, however, not until the concentration is increased to 0.20 pro mille. The phenylhydrazin reaction in the form described, as will be seen, is a very sensitive reaction,—much more sensitive than the reduction reactions commonly employed.

From the experiments on the fermentable sugar concentration of normal urine it appears that it may vary within rather wide limits and after a meal of bread, it may frequently exceed 1 pro mille, but as a rule it lies between 0.2 and 0.4 pro mille. In spite of this relatively large amount of fermentable sugar in the urine the phenylhydrazin reaction was always negative, as stated above. Since, however, urine usually requires only 0.10 pro mille glucose to be added to it in order that it shall react positively, this fact is in direct opposition to the assumption that the fermentable substance in normal urine is glucose.

It might, however, be thought that glucose constituted only a part of the fermentable substance in normal urine, and the concentration was too low to give a positive phenylhydrazin reaction. A series of experiments was therefore undertaken in which the fermentable substance was concentrated by evaporating the urine, and then the phenylhydrazin test was applied. A number of samples of urine were evaporated to different amounts varying from a half to a tenth of their volume, a little acetic acid (a couple of drops 50% glacial acetic acid to 50 cc. urine) being added, to be certain the reaction was acid, after which the phenylhydrazin test was performed. The result was that glucosazone could not be demonstrated in any of the evaporated samples of urine. Microscopically, practically the same forms were found in the urine as before evaporation, namely "grooved cakes," and a few rosettes, but in greater numbers than before evaporation.

The causes why glucosazone could not be demonstrated in the evaporated urine may be many. The glucose present might be destroyed in the process of evaporation, or the concentrated urine might hinder the formation of the glucosazone crystals. The reducing power of a number of samples of urine was there-

fore estimated by the previously described method of Benedict and Osterberg, before and after evaporation to one-tenth its original volume, with the result that the power of reduction in all the samples remained unchanged. Similarly there was no alteration in the urine to which 0.1-0.2 pro mille glucose was added before evaporation. In these urines the phenylhydrazin reaction was positive before evaporation, negative in the samples evaporated to 1/10th volume, but again positive when the evaporated urine was diluted with water to the original volume. This was proof that glucose is not destroyed during evaporation, but that it was the concentration of the urine that prevented the formation of the glucosazones.

To study further the influence of the concentration on the sensitiveness of the phenylhydrazin reaction, some samples of urine were evaporated to one-tenth volume and then a weighed quantity of glucose added. It was found that 0.5-1 pro mille glucose or even more had to be added to the concentrated urine before typical glucosazones were formed.

With increasing concentration of the urine, therefore, the sensitiveness of the phenylhydrazin reaction diminishes so much that it is not right to conclude there is no glucose in normal urine when the reaction is negative in an evaporated sample.

Neuberg<sup>63</sup> has already shown that urea, ammonium salts, etc., can hold a certain amount of glucosazone in solution, so that the phenylhydrazin reaction is less sensitive in urine than in watery solutions of glucose. As my experiments point in the same direction, I attempted to prepare glucosazone from evaporated urine in which the urea and other nitrogenous bodies had been precipitated. The filtrate from urine that had been treated with Pâtein's fluid and Zn-HCl was used, in accordance with the method previously described. The control tests, referred to earlier in this paper, it will be remembered, had proved that no sugar was lost in the process. HCl was added drop by drop to the filtrate till the reaction was weakly acid, and then it was evaporated to one-tenth volume, and the phenylhydrazin reaction carried out. Either numerous large "grooved cakes" or large osazone crystals were formed, which, however, consisted of coarse needles and were irregularly composed, but typical glucosazones were never produced. In order to test whether the evaporation destroyed the glucose or whether the evaporated filtrate prevented the



formation of the glucosazone, 0.05 pro mille glucose was added to the filtrate in many of the experiments. After evaporating it to one-tenth volume typical glucosazone crystals appeared. The reaction likewise was positive when 0.5 pro mille glucose was added to the filtrate after the evaporation.

Besides precipitating with Patein's fluid an attempt was made to treat the urine with blood charcoal and acetic acid as recommended by Andersen,<sup>64</sup> in which process, according to him, the sugar is not adsorbed by the charcoal. A number of samples of urine were treated in this manner, and after acidity of the filtrate had been reduced with solid sodium carbonate or a 20% solution of sodium hydroxide to a weakly acid reaction, it was evaporated to one-tenth volume. The phenylhydrazin reaction produced only numbers of large "grooved cakes," but not typical glucosazones.

Lastly a number of experiments were made in which the urine was shaken with bone charcoal prepared by Benedict's method (see above), and then filtered and evaporated. The phenylhydrazin reaction, however, gave the same result as after treating the urine with Patein's fluid and blood charcoal, namely, only large "cakes," and rosettes consisting of coarse needles, but no glucosazone.

It was not possible, therefore, to prepare glucosazone from evaporated urine, or urine whose nitrogenous constituents were for the most part removed, with subsequent evaporation of the urine.

Some experiments were next performed in *fermented urine* before and after the addition of definite amounts of glucose.

About 50 samples of urine from healthy persons were examined with the phenylhydrazin reaction before and after fermentation for 24, or more usually 48 hours, in the thermostat. The result was that before fermentation "physiological osazones" were found in varying quantities in all the samples, but after fermentation they could not be demonstrated, only some small "cakes" being found, while before fermentation numerous large cakes and rosettes were present. These investigations show that the *physiological osazones are due to the presence of carbohydrates in the urine.*

After fermentation the urine was decanted from the yeast and boiled for about 10-15 minutes to destroy the yeast cells and



enzyme, after which it was made up to the original volume with distilled water. Varying amounts of glucose were added to the urine so treated, and the phenylhydrazin reaction carried out. On microscopic examination the characteristic "grooved cakes," commonly observed in normal urine, were never found. The glucosazones occurred, as a rule, in their typical form without any preliminary stages. Irregularly formed osazones were only present in a few samples.

In these experiments it was found that typical glucosazones were formed when 0.2-0.3 pro mille glucose was added to the fermented urine; in other words, the sensitiveness of the reaction was only slightly less in fermented than in non-fermented urine.

As previously mentioned in describing the technique, fermentation in urine sometimes failed, and therefore its reducing power before and after the fermentation was controlled by two experiments and the amount of fermented sugar compared with the amount of glucose that had to be added to fermented urine to make the phenylhydrazin reaction positive.

*Experiment No. 156.*

Urine No. 1, from a healthy person, spec. gravity 1020, no albumin,  
Benedict's reaction —.

Total sugar: 1.20 pro mille.

Fermented after 24 hrs. : 0.39 pro mille

" " 48 " : 0.66 " "

—————Phenylhydrazin reaction—————

Urine					Before the fermentation	After 24 hrs. fermentation	After 48 hrs. fermentation
Urine + 0.05 pro mil. glucose :					—	—	—
"	+ 0.10	"	"	" :	+	—	—
"	+ 0.20	"	"	" :	+	+	+
"	+ 0.30	"	"	" :	+	+	+
"	+ 0.40	"	"	" :	+	+	+
"	+ 0.60	"	"	" :	+	+	+

*Experiment No. 157.*

Urine No. 2, from a healthy person, spec. gravity 1018, no albumin,  
Benedict's reaction —.

Total sugar : 1.10 pro mille  
Fermented after 24 hrs. : 0.40 pro mille  
" " 48 " : 0.70 " "

					————Phenylhydrazin reaction————		
Urine					Before the fermentation	After 24 hrs. fermentation	After 48 hrs. fermentation
Urine	+	0.05	pro mil.	glucose :	—	—	—
"	+	0.10	" "	" :	+	—	—
"	+	0.20	" "	" :	+	+	—
"	+	0.30	" "	" :	+	+	+
"	+	0.40	" "	" :	+	+	+
"	+	0.60	" "	" :	+	+	+

These experiments confirm the results previously referred to, which showed that the quantity of glucose, which must be added to fermented urine to make the phenylhydrazin reaction positive, was only slightly greater than the amount necessary before fermentation. The experiments further prove that if we replace the fermented sugar with the same amount of glucose or only a fraction of it, the phenylhydrazin reaction becomes positive.

These two experiments show, what was highly probable from the above-mentioned experiments on evaporated urine and the other experiments with fermented urine, that *the greater portion of the fermentable carbohydrates occurring in normal urine is not glucose*. But they do not prove that glucose does not occur in normal urine, because traces—less than about 0.05 pro mille—can hardly be detected by the method used. A point which is to a certain extent in favor of the existence of minimal amounts of glucose in the urine of healthy persons, is, as mentioned before, that some more glucose must be added to fermented urine than to unfermented urine, to produce typical glucosazone crystals. This, however, can be explained on the ground that the spherical or semi-spherical glucosazones develop as a superstructure on the physiological osazones, which is not improbable when it is remembered that these glucosazone forms were not found in solutions of glucose in water or in fermented urine to which sugar had been added, but only in unfermented urine containing sugar.

*Resume and Discussion.*

Benedict and Osterberg's method for determining the physiological sugar excretion proved not to be reliable, and therefore a few changes in the mode of procedure were made by means of which the amount of glucose added to urine could be accurately estimated.

A series of experiments showed that glucose added to urine was fermented very rapidly, even in minimal concentrations. The normal carbohydrate of the urine, on the other hand, was fermented comparatively slowly, the process usually not being ended before the lapse of 48 hours at 37°. Of great importance in fermentation is the hydrogen ion concentration of the urine, the optimum of which is roughly between pH 5.5 and 6.8.

In a series of healthy persons, a number of "transition cases," and a number of diabetics, the fermentable carbohydrate of the urine was estimated under various conditions and also its relation to clinical glycosuria and the blood sugar.

In the *healthy* cases it was found that the "total sugar" (Benedict and Osterberg) in 7 men fluctuated during 37 days between 450 and 1320 mg., the mean being 871 mg., while the excretion of "fermentable sugar" varied from 180 to 710 mg., with a mean of 368 mg. The sugar excretion was least in the morning before breakfast. The absorption of more water with its consequent considerable increase in diuresis, and the administration of sodium bicarbonate, was attended in a few experiments by increased sugar excretion, but in others it remained unchanged. Dilute hydrochloric acid had no effect on the sugar excretion.

The blood sugar concentration was not altered in healthy persons by alkali or acid.

Mixed food produced a marked increase in the sugar excretion and some increase in the blood sugar.

Of single articles of food, bread almost always occasioned an increased sugar excretion, the more pronounced the coarser the bread. The concentration of fermentable sugar in the urine rose especially after taking brown bread, and was often greater than 1 pro mille (maximum 1.8 pro mille). The greatest excretion took place, as a rule, in the 3rd hour after the meal. Benedict's qualitative sugar reaction and Almen's reaction were very often positive, particularly after taking brown bread, but the phenylhy-



drazin reaction, when it was employed, was negative. The ash of brown bread, however, had no influence on the sugar excretion.

After bread meals the blood sugar concentration showed some rise, but, in contrast to what happened to the urine sugar, it was greatest after taking fine bread, and less after brown bread. The maximum blood sugar concentration was always found in the first hour following the meal.

Two experiments with 300 gm. roast meat gave rise to a trifling increase in the sugar excretion, while 4 other experiments with meat, cabbage and soup were not accompanied by any increase.

The administration of glucose, as was to a certain extent already obvious from the experiments with single articles of food, showed that there was no relation between the blood sugar and the normal urine sugar, as the blood sugar could fluctuate within very wide limits, both as hyperglycemia and as hypoglycemia, without having any effect on the physiological sugar excretion. Only when the blood sugar transgressed the threshold was the fermentable sugar increased. In a few experiments, however, an increase in the urine sugar was demonstrable at a rather lower degree of hyperglycemia than that which just produced clinically detectable glycosuria, which can only be due to the fact that the quantitative method is better suited than the qualitative for demonstrating that the blood sugar has crossed the renal threshold.

The position of the renal threshold varied in 25 healthy persons\* between 114 and 216. In 3 cases where the threshold, with a good deal of latitude, could be determined in the rising and falling parts of the blood sugar curve, it was in two cases lower in the falling than in the rising portion, while in one case no difference could be found.

In all the 25 healthy persons investigated, the fasting blood sugar concentration was less than 110 mg. per 100 cc. blood. After taking 50 gm. glucose dissolved in 200 cc. water a blood sugar rise occurred, which varied greatly both in different individuals and in the same person. The greatest blood sugar concentration was 216 mg. In 2 hours' time the blood sugar in every case had again fallen below 110.

\* The two medical students, Nos. 16 and 20, who were described among the "transition cases," are here included. As they felt quite well and their intermittent glycosuria was discovered in these tolerance tests, and as there is no evidence that a low threshold is pathological (see later), there is justification in counting them among the "healthy." Apart from these two, the threshold in the remaining 23 was between 140 and 216.

In 6 of the 25 healthy persons glycosuria occurred after taking 50 gm. glucose dissolved in 200 cc. water.

There was no difference in the results of the tolerance tests done in the morning on a fasting stomach and those done in the afternoon, 3-4 hours after the last meal.

As glycosuria is dependent upon two factors, the height of the blood sugar rise and the position of the renal threshold, the first of which may vary greatly in similar experiments in the same person, while the second, as far as is known, is no indication of the condition of the carbohydrate metabolism—it is a bad measure of the carbohydrate tolerance. On the other hand, the *duration of the hyperglycemia*, as is shown by previous investigations as well as the present ones, is a good measure of tolerance. A blood sample taken before and 2 hours after the administration of 50 gm. glucose, dissolved in 200 cc. water, will be sufficient to gauge the carbohydrate metabolism in the majority of cases. If both samples are below 110, this is in favor of normal tolerance, and conversely, higher values point to hypofunction of the carbohydrate metabolism.

By injecting a 50% solution of glucose in water intravenously, the blood sugar rise occurred much earlier and was much sooner over than by oral administration. A specially low threshold, such as Rosenberg,<sup>49</sup> and Nonnenbruch and Szyszka<sup>48</sup> found by intravenous injection, could not be demonstrated in the present experiments.

In normal persons no change in the carbohydrate tolerance could be detected during digestion, as Benedict and his pupils<sup>19</sup> reported. Acid and alkali also had no effect on the tolerance for carbohydrates.

Of 8 "*transition cases*," 6 only showed evidence of a low renal threshold. In one the threshold was so low that glycosuria was constantly present. The blood sugar rise in these 6 was the same as in healthy persons. In the other 2, in addition to the low threshold, there was a higher blood sugar rise after glucose than is usually found in healthy persons. One of them, No. 61, at the second examination also had an increased fasting blood sugar in the morning, while the other, No. 64, had a marked diabetic taint in his family.

In one case, No. 20 (see experiments 121 and 122), the position of the threshold varied during the rising part of the blood sugar



curve, and in this individual, as also in Nos. 61 and 64, the threshold was considerably lower during the falling than the rising part of the blood sugar curve.

In all the "transition cases," the glycosuria was intimately bound up with the taking of carbohydrate, as one would expect if the glycosuria is entirely or mainly due to a low threshold. But this dependence only became prominent when the urine was collected for short intervals. When it was collected for longer periods, for example, daily, the difference in the sugar excretion on a carbohydrate-free and carbohydrate-rich diet was very slight, the reason being that after carbohydrate the blood sugar rise was very short and consequently the absolute amount of excreted sugar was small, so that when contained in the 24 hours' urine it only slightly increased the sugar concentration.

The reason that it is often considered characteristic of "renal diabetes" or "diabetes innocens" that carbohydrate has no effect, or very little, on the sugar excretion (Motzfeldt,<sup>37</sup> Graham,<sup>64</sup> and many others), is doubtless due to the fact that the urine was collected during too long periods (several hours up to 24).

In clinically interpreting the 8 "transition cases," it must be remembered that none of the many cases of glycosuria depending on a low threshold, which have been described in the literature, have passed over into diabetes. My own investigations into the threshold in healthy or apparently healthy persons also seem to show that a low renal threshold for glucose is fairly common. Both these facts indicate that a low threshold is not to be looked upon as the first stage of diabetes, but as a condition which has hardly any pathological significance.

It is more difficult to decide in what light two cases, Nos. 61 and 64, should be regarded, both of which showed other symptoms accompanying the low threshold which deviated from the normal, namely, a high blood sugar rise after glucose and a certain amount of fasting hyperglycemia in the morning. Both these symptoms might be in favor of diabetes, but as the blood sugar rise after glucose was over in two hours in each of them and the fasting hyperglycemia was only present on one day, it is impossible to decide whether these two ought to be regarded as incipient or slight cases of diabetes, or as innocent glycosuria.

The same amount of fermentable sugar was usually found in the 24 hours' urine of clinically sugar-free diabetics as in the



case of healthy persons, and the sugar excretion was not influenced, with the possible exception of one case, by a change of diet when the renal threshold was not exceeded. With the commencement of clinical glycosuria the fermentable sugar of the urine was suddenly much increased. This sudden and marked increase was—with simultaneous polyuria—often demonstrable one or two days before the qualitative sugar reactions became positive and could also be detected a few days after the clinical glycosuria had disappeared, after which the fermentable sugar in the urine again fell suddenly to its previous level.

Pathological sugar excretion in diabetics can therefore be detected by Benedict and Osterberg's method where the qualitative methods fail.

Changes in the blood sugar concentration produced by the administration of glucose did not cause any alteration in the urine sugar in diabetics when the threshold was not transgressed. Nor had acids and alkalis, which were given with glucose, any effect on the urine sugar or on the blood sugar rise induced by glucose.

Single articles of food, such as cabbage, bacon and meat did not produce an increase in the urine sugar without a simultaneous blood sugar rise. Even 500 gm. cabbage did not affect the amount of urine sugar, so that the work of digestion, as such, in diabetics as in healthy persons, has no influence on the sugar excretion.

In quantities of 300 gm., meat caused an increase in the blood sugar concentration in all the experiments with diabetics. Moreover, a meat diet lowered the renal threshold in two diabetics investigated. On the other hand, the digestion of meat had no effect on the blood sugar rise produced by glucose.

Investigations into the nature of the physiological urine sugar showed that at any rate the major portion of the fermentable carbohydrate of the urine is not glucose, and that it must be regarded as doubtful whether glucose is a normal urinary constituent at all.\*

We may, therefore, distinguish between two kinds of sugar excretion in the urine:

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\* The reason that Baisch succeeded in preparing glucosazone from normal urine may be that closely related carbohydrates may have been transformed into glucose by the many chemical processes necessitated by the method which Baisch employed.

1. *Physiological sugar excretion*, which has no relation to the amount of glucose in the blood and which comprises sugars whose nature is not known, but which probably do not include glucose. After meals consisting of bread, as well as in urine which is concentrated, these physiological sugars may occur in such quantities that the reduction reactions commonly employed are positive.

2. *Pathological sugar excretion*, caused by the passage of the glucose of the blood into the urine when the blood sugar concentration exceeds the renal threshold.

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### ARTICLE A-3.

## CORRECTIONS FOR GAS VOLUMES FOR ALTITUDES 700 TO 600 MM.

*A table combining the corrections for barometric pressure, room temperature, brass scale expansion and vapor tension.*

BY HARRY GAUSS, M.S., M.D., Denver, Colorado.

The following table of logarithmic corrections has been prepared for the reduction to standard conditions of gas volumes for the higher altitudes from 700 to 600 mm., and between the temperature ranges from 15° to 32° Centigrade.

The immediate need for these corrections arose in our studies in basal metabolism conducted as a clinical laboratory procedure, also, in our studies on the influence of carbon dioxide on the tubercle bacillus.<sup>1, 2</sup> Both in the clinical laboratory procedure and in the research problem an analysis is required of air after respiration by the patient in one case and by the bacteria in the other. The principle of respiration is physiologically comparable in both.

In making the gas analysis calculations, the need arises for a set of tables combining the several variables into one factor to facilitate the reduction to standard conditions. It was found that numerous tables of this kind exist, but that they all contemplate approximate sea level conditions. Boothby and Sandiford<sup>3</sup> have prepared a set of these tables for the barometric ranges of 700 to 780 mm. and between the temperature range of 15° to 32°. We, in Denver and other localities, having a barometric range beyond those commonly considered find ourselves confronted with the necessity of making the calculations by the more laborious method of applying each of the several corrections individually, hence, the need for this compilation. If a similar table exists elsewhere, we have been unable to find it.

The need for these corrections exists not only in medicine but in numerous other technical industries and schools of Colorado

and elsewhere. It will be of great service to the physician and of urgent need to those interested in basal metabolism determinations in the Rocky Mountain region.

The four factors considered here—(1) correction for barometric pressure, (2) correction for room temperature, (3) brass scale expansion and vapor tension were all obtained from Landolt-Bornstein, *Physikalisch-Chemische Tabellen*,<sup>4</sup> except a part of the corrections for brass scale expansion which we were unable to find for the altitudes 640 to 600, and for which we were thrown upon our own resources and chose the convenient method of interpolation instead of applying a formidable looking formula; for example:

Brass scale Corrections in mm. (Landolt-Bornstein)									
	640	650	660	670	680	690	700	710	720
at 15°C.	1.56	1.59	1.61	1.64	1.66	1.69	1.71	1.74	1.76
intervals	3	2	3	2	3	2	3	2	

Assuming that the rate of expansion between 640 and 600 continues at this same ratio, it is easy enough to extend the interval figures down to 600, and put in the figures corresponding to the expansion in mm.; thus—

Bar. pressure in mm.	600	610	620	630	640	650	660
interval is		3	2	3	2	3	2
at 15°C. brass scale							
correction becomes	1.46	1.49	1.51	1.54	1.56	1.59	1.61

By a similar manner of interpolation the brass scale corrections for the barometric pressures 600 to 640 between the temperature ranges 15° to 32°C. were obtained and utilized. However, since it is unwise to assume too much, a few calculations were made by another method as a check. Utilizing the data in the Smithsonian Physical Tables<sup>5</sup> and calculating the results, R. G. Gustafson of the University of Denver obtained figures quite close to those mentioned, certainly within the limits of most experimental problems, as shown in the following tables:

Calculated Brass Scale Corrections for 15°C.

Bar. pressure in mm.	600	610	620	630	640	650	660
By interpolation (Gauss)	1.46	1.49	1.51	1.54	1.56	1.59	1.61
From data in Smithsonian tables (Gustafson)	1.461	1.488	1.505	1.538	1.56	1.582	1.61



TABLE I (page A)

Log Factor for Reducing Volume of Gases to Standard Temperature and Pressure,  
including Corrections for Vapor Tension and Brass Scale Expansion.

	600	601	602	603	604	605	606	607	608	609	610	611	612	613	614	615	616	617	618	619	620	621	622
15.0	1.86362	86463	86509	86583	86657	86730	86803	86876	86949	87022	87095	87168	87241	87314	87386	87558	87530	87602	87673	87745	87817	87889	87960
15.5	86252	86326	86400	86473	86547	86620	86693	86766	86839	86912	86985	87058	87131	87204	87275	87348	87420	87492	87564	87636	87708	87780	87851
16.0	86141	86215	86289	86363	86436	86510	86583	86656	86729	86802	86875	86948	87021	87094	87166	87238	87310	87382	87454	87526	87598	87670	87741
16.5	86030	86104	86178	86251	86325	86398	86472	86545	86618	86691	86764	86837	86910	86983	87056	87128	87200	87272	87344	87416	87488	87560	87631
17.0	85917	85991	86065	86139	86213	86286	86360	86434	86506	86580	86653	86725	86798	86871	86944	87016	87088	87160	87232	87304	87376	87447	87519
17.5	85805	85879	85952	86026	86100	86174	86247	86321	86394	86467	86540	86613	86685	86758	86831	86904	86976	87048	87120	87191	87263	87335	87407
18.0	85689	85764	85838	85911	85985	86059	86132	86206	86280	86352	86426	86499	86572	86645	86718	86791	86863	86935	87008	87079	87151	87223	87294
18.5	85574	85649	85723	85797	85870	85944	86018	86092	86165	86238	86311	86384	86457	86530	86602	86675	86748	86820	86893	86965	87037	87109	87180
19.0	85458	85533	85607	85681	85755	85828	85902	85976	86050	86123	86196	86270	86342	86415	86488	86560	86633	86706	86778	86850	86922	86993	87065
19.5	85341	85415	85490	85564	85638	85711	85785	85859	85932	86006	86079	86153	86226	86299	86372	86444	86517	86590	86661	86734	86806	86878	86949
20.0	85223	85298	85372	85447	85521	85594	85668	85741	85815	85889	85962	86035	86109	86182	86255	86328	86400	86473	86546	86618	86690	86762	86834
20.5	85104	85179	85254	85328	85403	85476	85550	85623	85697	85771	85844	85918	85991	86064	86137	86210	86282	86355	86428	86500	86572	86645	86717
21.0	84984	85059	85134	85208	85283	85357	85430	85504	85577	85651	85724	85798	85871	85945	86018	86091	86163	86236	86309	86382	86453	86526	86598
21.5	84862	84937	85012	85087	85161	85236	85310	85383	85457	85531	85604	85678	85752	85824	85898	85970	86043	86116	86189	86262	86334	86406	86478
22.0	84740	84815	84890	84965	85040	85114	85188	85262	85336	85410	85483	85556	85630	85704	85776	85850	85922	85995	86068	86140	86213	86286	86358
22.5	84617	84691	84766	84841	84915	84990	85065	85138	85212	85286	85360	85434	85508	85581	85654	85728	85800	85873	85945	86018	86091	86163	86235
23.0	84493	84567	84642	84717	84791	84866	84941	85016	85089	85163	85237	85310	85384	85457	85531	85604	85677	85750	85822	85895	85968	86041	86114
23.5	84365	84441	84515	84590	84665	84739	84814	84888	84963	85036	85110	85184	85258	85332	85405	85479	85552	85625	85698	85770	85843	85916	85988
24.0	84239	84315	84390	84465	84539	84614	84688	84763	84838	84911	84985	85058	85132	85206	85279	85353	85426	85500	85572	85645	85718	85790	85863
24.5	84112	84187	84263	84337	84412	84487	84561	84636	84710	84785	84859	84932	85006	85079	85153	85226	85300	85373	85447	85520	85592	85665	85738
25.0	83983	84058	84133	84209	84283	84358	84432	84507	84582	84656	84731	84804	84878	84952	85025	85099	85172	85246	85319	85392	85465	85537	85610
25.5	83851	83927	84002	84077	84152	84227	84301	84376	84451	84525	84599	84674	84747	84821	84894	84968	85042	85116	85190	85262	85336	85409	85481
26.0	83718	83794	83870	83944	84020	84095	84170	84244	84319	84394	84468	84543	84617	84690	84764	84837	84911	84985	85058	85131	85205	85278	85350
26.5	83585	83660	83736	83812	83886	83962	84036	84111	84186	84260	84335	84409	84484	84558	84631	84705	84778	84852	84926	84999	85072	85146	85219
27.0	83449	83525	83601	83677	83753	83827	83903	83978	84052	84127	84201	84276	84350	84424	84498	84572	84645	84719	84792	84866	84939	85012	85085
27.5	83313	83389	83465	83540	83616	83691	83766	83841	83916	83990	84065	84139	84214	84289	84363	84437	84510	84584	84658	84731	84805	84878	84951
28.0	83175	83252	83327	83403	83478	83554	83629	83704	83779	83854	83938	84003	84077	84152	84227	84301	84375	84448	84522	84596	84669	84732	84816
28.5	83048	83125	83201	83276	83352	83427	83503	83577	83653	83727	83802	83877	83952	84026	84101	84175	84249	84322	84396	84469	84543	84617	84690
29.0	82892	82969	83046	83122	83198	83273	83349	83424	83499	83574	83649	83724	83799	83873	83948	84022	84097	84170	84244	84317	84391	84465	84539
29.5	82751	82827	82903	82979	83055	83130	83206	83281	83357	83432	83508	83582	83657	83731	83806	83880	83955	84031	84103	84177	84251	84324	84398
30.0	82605	82682	82759	82834	82911	82986	83062	83137	83213	83288	83363	83438	83515	83588	83663	83738	83812	83887	83961	84035	84109	84182	84256
30.5	82459	82535	82611	82688	82763	82840	82915	82991	83067	83143	83218	83293	83368	83443	83517	83592	83667	83742	83817	83892	83965	84039	84112
31.0	82310	82388	82463	82540	82616	82692	82768	82844	82919	82995	83071	83147	83221	83297	83371	83446	83520	83595	83670	83745	83819	83893	83966
31.5	82160	82237	82314	82390	82466	82543	82618	82695	82770	82846	82922	82998	83073	83148	83223	83298	83372	83447	83521	83596	83670	83745	83819
32.0	82009	82085	82162	82240	82315	82392	82468	82543	82620	82696	82771	82847	82923	82998	83073	83148	83223	83297	83372	83446	83521	83596	83671



TABLE I (page B)

Log Factor for Reducing Volume of Gases to Standard Temperature and Pressure,  
including Corrections for Vapor Tension and Brass Scale Expansion.

	623	624	625	626	627	628	629	630	631	632	633	634	635	636	637	638	639	640	641	642	643	644	645
15.0	88031	88102	88173	88244	88315	88386	88456	88526	88597	88667	88736	88807	88877	88947	89017	89086	89156	89226	89294	89363	89432	89501	89570
15.5	87922	87993	88064	88135	88206	88277	88347	88417	88488	88558	88628	88698	88768	88838	88908	88977	89047	89116	89185	89254	89323	89392	89461
16.0	87812	87883	87954	88026	88097	88168	88239	88308	88379	88449	88519	88589	88659	88729	88799	88869	88938	89007	89077	89143	89215	89284	89353
16.5	87702	87773	87844	87916	87987	88058	88129	88199	88269	88339	88409	88479	88549	88619	88689	88759	88828	88898	88968	89037	89106	89175	89244
17.0	87591	87662	87733	87804	87876	87946	88017	88088	88158	88228	88298	88368	88439	88509	88579	88649	88719	88788	88857	88927	88996	89065	89134
17.5	87479	87551	87622	87693	87764	87835	87906	87977	88047	88117	88187	88257	88328	88398	88468	88538	88608	88677	88747	88816	88885	88954	89024
18.0	87365	87437	87508	87579	87650	87721	87792	87863	87934	88004	88075	88145	88215	88285	88356	88426	88495	88565	88634	88703	88773	88842	88911
18.5	87252	87324	87395	87466	87537	87608	87679	87750	87821	87891	87961	88031	88102	88172	88242	88312	88382	88451	88520	88590	88660	88729	88798
19.0	87137	87209	87280	87352	87423	87494	87565	87636	87707	87778	87848	87919	87989	88059	88129	88199	88269	88339	88409	88478	88548	88618	88685
19.5	87021	87093	87165	87236	87307	87378	87450	87521	87591	87662	87733	87803	87873	87944	88014	88083	88154	88224	88293	88363	88432	88502	88571
20.0	86905	86977	87049	87120	87192	87263	87333	87405	87476	87547	87617	87687	87758	87828	87898	87968	88038	88108	88178	88248	88317	88386	88456
20.5	86788	86860	86932	87003	87075	87146	87217	87288	87360	87430	87501	87572	87642	87712	87783	87852	87922	87993	88063	88132	88202	88271	88340
21.0	86669	86741	86813	86885	86957	87028	87099	87170	87241	87312	87383	87453	87523	87594	87664	87734	87804	87875	87944	88014	88084	88153	88223
21.5	86550	86622	86693	86765	86837	86908	86980	87050	87121	87192	87263	87334	87405	87475	87545	87616	87686	87756	87827	87897	87966	88036	88104
22.0	86431	86502	86574	86645	86717	86789	86860	86932	87002	87073	87144	87215	87285	87356	87426	87496	87567	87636	87706	87777	87847	87916	87986
22.5	86307	86379	86450	86523	86595	86666	86738	86810	86880	86951	87022	87092	87164	87235	87305	87373	87446	87516	87586	87656	87726	87795	87865
23.0	86185	86258	86330	86401	86473	86544	86616	86688	86759	86830	86901	86972	87043	87113	87184	87254	87325	87396	87465	87535	87605	87675	87745
23.5	86061	86132	86205	86277	86348	86420	86491	86563	86635	86706	86777	86848	86919	86990	87061	87131	87201	87272	87342	87412	87482	87552	87622
24.0	85936	86007	86080	86152	86223	86295	86367	86439	86510	86582	86653	86724	86795	86866	86937	87008	87078	87148	87219	87289	87359	87429	87499
24.5	85810	85882	85954	86026	86098	86170	86242	86314	86385	86457	86528	86599	86670	86741	86812	86883	86954	87024	87094	87164	87234	87304	87375
25.0	85683	85745	85828	85899	85972	86044	86115	86187	86258	86330	86402	86473	86544	86615	86686	86757	86827	86898	86968	87039	87109	87179	87249
25.5	85554	85626	85699	85772	85843	85916	85987	86059	86131	86202	86274	86345	86417	86488	86559	86630	86700	86771	86841	86911	86981	87051	87121
26.0	85423	85495	85568	85640	85712	85785	85857	85928	86000	86072	86143	86215	86286	86357	86428	86500	86571	86641	86712	86782	86852	86922	86993
26.5	85291	85364	85436	85509	85581	85653	85725	85797	85868	85940	86012	86084	86156	86227	86298	86369	86440	86511	86582	86653	86723	86793	86864
27.0	85158	85231	85303	85376	85449	85522	85594	85666	85738	85810	85881	85953	86024	86096	86167	86238	86309	86380	86451	86522	86593	86663	86733
27.5	85024	85097	85170	85242	85315	85388	85460	85532	85604	85676	85747	85819	85891	85963	86034	86105	86176	86247	86318	86389	86460	86530	86600
28.0	84889	84962	85035	85107	85180	85252	85325	85397	85469	85541	85613	85685	85756	85828	85899	85971	86043	86113	86184	86255	86326	86396	86467
28.5	84763	84837	84909	84982	85054	85127	85200	85273	85344	85416	85488	85560	85632	85703	85775	85847	85919	85989	86060	86131	86202	86273	86344
29.0	84613	84685	84758	84831	84904	84977	85049	85122	85194	85267	85339	85411	85482	85554	85625	85697	85768	85840	85911	85982	86053	86125	86196
29.5	84472	84545	84618	84691	84764	84837	84910	84983	85055	85128	85200	85272	85343	85415	85487	85559	85630	85702	85773	85844	85915	85986	86057
30.0	84330	84404	84477	84550	84623	84696	84768	84841	84913	84986	85059	85131	85203	85275	85346	85418	85490	85562	85633	85704	85775	85846	85917
30.5	84186	84259	84333	84406	84479	84552	84627	84698	84771	84843	84916	84988	85060	85132	85204	85276	85348	85419	85491	85562	85634	85705	85776
31.0	84040	84111	84187	84260	84334	84407	84480	84553	84626	84698	84771	84843	84916	84987	85060	85132	85204	85275	85347	85418	85490	85562	85633
31.5	83893	83966	84040	84113	84187	84260	84333	84407	84480	84552	84625	84697	84770	84842	84914	84987	85059	85130	85202	85273	85345	85416	85489
32.0	83744	83818	83891	83965	84038	84112	84186	84259	84332	84405	84477	84550	84622	84695	84768	84840	84912	84984	85056	85127	85199	85271	85342



(TABLE I (page C))

Log Factor for Reducing Volume of Gases to Standard Temperature and Pressure,  
including Corrections for Vapor Tension and Brass Scale Expansion.

	646	647	648	649	650	651	652	653	654	655	656	657	658	659	660	661	662	663	664	665	666	667	668	669
15.0	1.89638	89706	89774	89842	89911	89979	90047	90115	90183	90251	90319	90386	90453	90520	90588	90655	90722	90789	90855	90922	90989	91055	91121	91187
15.5	89530	89599	89667	89735	89803	89871	89939	90007	90075	90145	90211	90278	90346	90413	90481	90547	90614	90681	90748	90814	90881	90947	91014	91080
16.0	89422	89491	89559	89627	89696	89764	89832	89900	89968	90036	90104	90172	90239	90306	90372	90440	90507	90573	90640	90707	90774	90841	90907	90973
16.5	89313	89382	89450	89518	89587	89655	89723	89791	89859	89927	89994	90061	90128	90196	90263	90331	90398	90464	90531	90598	90665	90732	90798	90864
17.0	89203	89272	89340	89408	89477	89545	89613	89681	89749	89817	89885	89953	90020	90088	90155	90223	90289	90356	90423	90490	90557	90624	90690	90756
17.5	89092	89161	89230	89298	89366	89434	89502	89570	89639	89707	89775	89843	89910	89977	90045	90112	90179	90246	90314	90380	90447	90514	90581	90646
18.0	88980	89049	89118	89186	89254	89323	89391	89459	89527	89596	89664	89732	89799	89866	89934	90002	90069	90136	90203	90270	90336	90403	90470	90536
18.5	88868	88936	89005	89074	89142	89210	89278	89346	89414	89483	89551	89619	89687	89754	89822	89889	89956	90024	90090	90157	90224	90291	90356	90425
19.0	88754	88824	88893	88961	89030	89098	89166	89235	89303	89371	89439	89507	89575	89642	89709	89777	89844	89911	89979	90046	90113	90180	90247	90313
19.5	88640	88709	88778	88847	88916	88984	89052	89121	89189	89257	89324	89393	89461	89528	89596	89663	89730	89798	89865	89932	89999	90066	90132	90199
20.0	88525	88593	88663	88732	88800	88869	88937	89005	89074	89142	89210	89279	89347	89414	89482	89549	89617	89684	89751	89819	89885	89952	90019	90085
20.5	88410	88478	88547	88617	88685	88754	88823	88891	88959	89028	89095	89163	89232	89299	89367	89435	89502	89569	89637	89704	89771	89838	89905	89971
21.0	88293	88362	88431	88500	88569	88637	88706	88773	88842	88911	88979	89047	89115	89183	89251	89318	89385	89453	89521	89588	89655	89722	89789	89856
21.5	88174	88243	88312	88381	88450	88519	88588	88657	88724	88793	88861	88929	88997	89066	89133	89201	89269	89336	89404	89471	89538	89605	89672	89739
22.0	88055	88124	88193	88262	88331	88401	88469	88538	88607	88674	88743	88812	88879	88947	89016	89083	89151	89219	89286	89354	89421	89488	89555	89622
22.5	87934	88004	88074	88143	88211	88281	88350	88418	88487	88555	88623	88692	88759	88827	88896	88964	89032	89100	89167	89234	89302	89368	89436	89503
23.0	87815	87884	87953	88023	88092	88160	88230	88298	88367	88436	88503	88572	88640	88708	88776	88845	88913	88980	89048	89115	89182	89249	89316	89384
23.5	87692	87761	87830	87899	87969	88038	88106	88176	88244	88313	88382	88449	88518	88586	88654	88722	88790	88858	88926	88993	89060	89128	89196	89262
24.0	87568	87638	87707	87777	87847	87915	87984	88054	88122	88191	88259	88327	88396	88465	88533	88600	88669	88737	88804	88872	88939	89007	89074	89141
24.5	87444	87514	87583	87652	87722	87792	87861	87929	87999	88068	88136	88205	88272	88341	88410	88477	88545	88614	88682	88750	88817	88884	88951	89019
25.0	87319	87389	87459	87528	87597	87666	87736	87805	87874	87943	88011	88080	88149	88217	88286	88354	88422	88489	88558	88626	88693	88761	88828	88895
25.5	87191	87261	87331	87400	87470	87539	87608	87677	87746	87815	87885	87954	88023	88091	88159	88228	88296	88364	88431	88500	88568	88635	88703	88769
26.0	87063	87133	87203	87273	87342	87411	87481	87550	87619	87688	87757	87826	87895	87964	88032	88100	88169	88237	88305	88372	88441	88509	88576	88644
26.5	86933	87003	87073	87143	87213	87282	87351	87421	87491	87560	87629	87698	87767	87835	87904	87971	88040	88108	88176	88244	88312	88380	88448	88515
27.0	86804	86874	86944	87014	87083	87153	87222	87291	87361	87431	87499	87568	87637	87706	87774	87843	87911	87979	88048	88117	88185	88253	88321	88389
27.5	86670	86741	86811	86880	86951	87021	87091	87161	87230	87298	87368	87436	87505	87574	87643	87711	87780	87848	87916	87985	88053	88121	88189	88257
28.0	86537	86608	86678	86748	86817	86887	86957	87027	87097	87165	87235	87304	87373	87442	87511	87580	87648	87717	87785	87854	87922	87990	88058	88126
28.5	86415	86485	86555	86626	86696	86765	86835	86905	86975	87044	87113	87182	87252	87320	87389	87458	87527	87595	87664	87732	87800	87868	87936	88003
29.0	86266	86337	86407	86477	86548	86617	86687	86757	86827	86896	86966	87034	87104	87174	87243	87311	87381	87449	87518	87586	87654	87722	87791	87858
29.5	86128	86198	86269	86339	86409	86479	86549	86619	86689	86758	86828	86898	86967	87036	87106	87174	87243	87312	87381	87449	87518	87585	87654	87722
30.0	85988	86059	86129	86199	86270	86340	86410	86480	86550	86620	86690	86759	86829	86898	86968	87037	87106	87174	87244	87312	87381	87449	87517	87585
30.5	85847	85918	85988	86059	86128	86199	86270	86340	86409	86479	86549	86619	86689	86759	86827	86897	86966	87035	87103	87173	87241	87310	87378	87446
31.0	85704	85775	85845	85916	85987	86057	86127	86197	86267	86337	86408	86478	86547	86617	86686	86755	86824	86894	86962	87031	87100	87169	87237	87306
31.5	85559	85630	85701	85772	85842	85913	85984	86054	86124	86195	86264	86334	86404	86474	86543	86613	86681	86751	86820	86889	86958	87028	87096	87165
32.0	85414	85485	85556	85626	85698	85768	85839	85909	85979	86049	86119	86189	86259	86329	86399	86460	86538	86607	86677	86746	86815	86883	86953	87021

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TABLE I (page D)

Log Factor for Reducing Volume of Gases to Standard Temperature and Pressure,  
including Corrections for Vapor Tension and Brass Scale Expansion.

	670	671	672	673	674	675	676	677	678	679	680	681	682	683	684	685	686	687	688	689
15.0	1.91254	91320	91386	91451	91517	91583	91649	91714	91779	91844	91910	91975	92039	92104	92169	92234	92298	92363	92427	92492
15.5	91147	91213	91278	91344	91410	91476	91542	91606	91672	91737	91803	91868	91933	91998	92062	92127	92192	92257	92321	92386
16.0	91039	91105	91171	91237	91303	91369	91435	91500	91565	91630	91695	91761	91826	91890	91955	92020	92085	92150	92215	92279
16.5	90930	90997	91063	91129	91195	91261	91327	91393	91458	91523	91588	91654	91719	91783	91848	91913	91978	92043	92108	92172
17.0	90823	90889	90955	91021	91087	91153	91219	91284	91350	91415	91480	91545	91611	91675	91740	91805	91870	91935	92000	92064
17.5	90731	90779	90845	90911	90977	91043	91109	91175	91241	91305	91371	91436	91501	91566	91631	91696	91761	91826	91891	91955
18.0	90602	90669	90734	90801	90867	90933	90999	91065	91131	91196	91261	91327	91391	91457	91522	91587	91651	91716	91781	91845
18.5	90591	90557	90623	90690	90756	90821	90887	90953	91019	91084	91150	91215	91281	91346	91411	91476	91540	91605	91670	91735
19.0	90380	90446	90512	90579	90645	90710	90776	90842	90908	90974	91040	91105	91170	91235	91301	91365	91430	91495	91560	91624
19.5	90266	90332	90398	90465	90532	90597	90663	90729	90794	90860	90926	90991	91056	91122	91187	91252	91317	91382	91447	91512
20.0	90152	90219	90285	90352	90418	90485	90550	90616	90682	90747	90813	90879	90944	91010	91075	91140	91205	91269	91334	91399
20.5	90038	90105	90172	90237	90304	90370	90437	90503	90569	90634	90700	90766	90831	90896	90962	91026	91092	91156	91221	91286
21.0	89923	89989	90056	90122	90189	90255	90321	90387	90453	90519	90585	90650	90716	90781	90846	90911	90977	91042	91107	91172
21.5	89805	89872	89939	90006	90072	90138	90204	90271	90337	90403	90468	90534	90600	90665	90730	90795	90860	90926	90990	91055
22.0	89689	89756	89822	89889	89956	90021	90088	90154	90220	90286	90352	90417	90483	90549	90614	90679	90744	90809	90875	90939
22.5	89570	89637	89704	89770	89837	89903	89969	90035	90102	90168	90234	90299	90365	90431	90496	90561	90627	90692	90758	90823
23.0	89451	89517	89584	89651	89718	89785	89851	89917	89983	90050	90116	90181	90247	90312	90378	90444	90508	90574	90639	90705
23.5	89330	89397	89463	89530	89596	89663	89730	89796	89862	89927	89994	90060	90125	90191	90257	90323	90389	90453	90519	90583
24.0	89209	89276	89342	89409	89476	89543	89609	89676	89742	89808	89874	89940	90006	90072	90138	90203	90269	90335	90399	90465
24.5	89086	89153	89220	89287	89354	89421	89487	89554	89620	89686	89752	89819	89885	89950	90016	90082	90147	90203	90277	90343
25.0	88963	89029	89096	89163	89230	89297	89364	89430	89497	89562	89629	89695	89761	89827	89897	89959	90024	90090	90156	90221
25.5	88837	88904	88971	89038	89105	89172	89239	89306	89373	89439	89505	89571	89637	89703	89769	89835	89901	89966	90032	90098
26.0	88711	88779	88846	88913	88980	89047	89113	89180	89246	89313	89379	89445	89511	89577	89644	89709	89775	89841	89906	89972
26.5	88582	88650	88716	88785	88852	88919	88985	89052	89119	89186	89253	89319	89385	89451	89517	89583	89649	89715	89781	89846
27.0	88456	88523	88590	88658	88724	88792	88858	88925	88991	89058	89124	89191	89257	89324	89390	89456	89522	89587	89653	89719
27.5	88325	88392	88459	88526	88594	88660	88728	88795	88862	88928	88995	89061	89128	89194	89260	89326	89393	89459	89525	89590
28.0	88193	88261	88328	88395	88463	88531	88597	88665	88731	88798	88864	88931	88997	89064	89130	89197	89263	89329	89395	89461
28.5	88072	88139	88207	88275	88342	88409	88477	88544	88611	88678	88745	88811	88878	88944	89011	89076	89143	89208	89275	89340
29.0	87926	87994	88062	88129	88197	88264	88332	88399	88466	88533	88600	88666	88733	88800	88866	88933	88999	89065	89131	89197
29.5	87790	87857	87925	87993	88061	88128	88195	88262	88330	88397	88465	88531	88598	88665	88731	88798	88865	88931	88997	89063
30.0	87654	87721	87789	87857	87925	87992	88059	88126	88194	88261	88328	88395	88462	88529	88596	88663	88729	88796	88862	88928
30.5	87514	87583	87650	87718	87786	87854	87922	87989	88056	88124	88191	88258	88325	88392	88458	88525	88591	88658	88725	88791
31.0	87373	87442	87510	87578	87646	87714	87782	87849	87917	87984	88052	88120	88186	88254	88320	88387	88453	88520	88586	88653
31.5	87233	87301	87369	87437	87505	87573	87641	87709	87776	87844	87910	87978	88045	88111	88179	88245	88312	88379	88445	88512
32.0	87090	87158	87225	87294	87362	87430	87497	87565	87633	87700	87768	87835	87903	87970	88037	88104	88171	88237	88304	88370



TABLE I (page E)

Log Factor for Reducing Volume of Gases to Standard Temperature and Pressure,  
including Corrections for Vapor Tension and Brass Scale Expansion.

	690	691	692	693	694	695	696	697	698	699	700
15.0	92555	92619	92684	92748	92812	92876	92939	93003	93066	93120	93193
15.5	92449	92513	92578	92642	92706	92770	92833	92896	92960	93023	93087
16.0	92343	92407	92471	92536	92600	92663	92727	92790	92854	92918	92981
16.5	92236	92300	92364	92428	92492	92556	92620	92683	92747	92810	92873
17.0	92128	92192	92256	92320	92385	92449	92513	92576	92639	92703	92767
17.5	92019	92084	92147	92211	92276	92340	92404	92468	92531	92594	92658
18.0	91910	91974	92038	92102	92166	92231	92294	92358	92422	92485	92549
18.5	91800	91864	91928	91993	92057	92121	92185	92249	92312	92376	92439
19.0	91689	91754	91817	91882	91946	92010	92074	92138	92202	92266	92328
19.5	91577	91641	91706	91770	91835	91898	91962	92027	92090	92154	92218
20.0	91464	91529	91594	91657	91722	91786	91849	91914	91978	92041	92105
20.5	91351	91416	91481	91545	91609	91674	91737	91801	91866	91929	91993
21.0	91236	91301	91366	91431	91495	91559	91624	91687	91751	91816	91879
21.5	91120	91185	91250	91314	91379	91443	91508	91572	91636	91701	91764
22.0	91004	91069	91134	91199	91263	91328	91391	91456	91520	91583	91648
22.5	90887	90952	91017	91081	91146	91211	91275	91340	91403	91467	91532
23.0	90770	90834	90899	90963	91028	91093	91157	91221	91286	91350	91414
23.5	90649	90714	90778	90843	90908	90973	91038	91103	91167	91231	91295
24.0	90530	90595	90660	90725	90789	90854	90918	90983	91048	91112	91177
24.5	90408	90473	90538	90603	90668	90733	90797	90862	90927	90991	91056
25.0	90286	90351	90417	90481	90546	90611	90675	90740	90805	90869	90933
25.5	90163	90229	90293	90359	90423	90488	90553	90617	90682	90746	90811
26.0	90037	90102	90168	90232	90296	90363	90428	90492	90557	90622	90686
26.5	89912	89976	90042	90107	90173	90238	90302	90367	90432	90496	90561
27.0	89784	89850	89915	89981	90046	90111	90176	90241	90305	90370	90434
27.5	89656	89721	89787	89851	89917	89982	90048	90112	90177	90241	90306
28.0	89526	89592	89657	89723	89787	89853	89918	89984	90048	90113	90177
28.5	89406	89472	89538	89603	89669	89734	89799	89864	89929	89994	90059
29.0	89263	89329	89395	89460	89526	89591	89656	89721	89786	89851	89916
29.5	89130	89195	89261	89326	89393	89457	89523	89587	89653	89717	89783
30.0	88994	89060	89126	89191	89257	89322	89388	89454	89519	89584	89649
30.5	88857	88923	88989	89055	89120	89186	89251	89319	89382	89447	89513
31.0	88718	88785	88850	88917	88982	89048	89113	89179	89244	89310	89374
31.5	88578	88644	88710	88776	88842	88908	88974	89040	89105	89171	89236
32.0	88437	88504	88570	88636	88702	88768	88834	88899	88965	89031	89097

The combined correction is then computed as follows:  
Example, for a barometric pressure of 632 mm. and a temperature of 23°C.

632.0 mm.

2.368 correction for brass scale expansion.

---

629.632

21.074 correction for vapor tension.

---

608.558 corrected barometric reading.

the correction to standard barometric pressure is obtained by the  $\log \frac{h}{760}$  which for 608.56 is .90349—1; and the correction to

standard temperature is obtained by the  $\log \frac{1}{1 + 0.00367 t}$  which

for 23°C. is .90349—1;

therefore .90349—1

.96481—1

---

.86830—1 which is the log for correction of a gas volume to standard conditions from 23°C. and 632 mm.

In a similar manner, the calculations were made for each mm. variation from 600 to 700 mm., and for each 0.5° from 15° to 32°C.; the results are given in the following tables:

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## EXPERIMENTAL STUDIES IN DIABETES

### Series V. Acidosis

#### 5. Ketosis in Eck fistula dogs

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It seems unnecessary to review the familiar experiments which support the widespread view, especially in German literature, that the acetone bodies are formed exclusively or predominantly in the liver. Perfusions of "surviving" organs, as in the well-known experiments of Embden,<sup>1</sup> cannot be considered to decide the question. The experiments to be reported in the present paper were prompted especially by the work of Fischler and Kossow,<sup>2</sup> who found that dogs with Eck fistulas excreted less acetone bodies than normal dogs, while dogs with reversed Eck fistulas excreted more than normal dogs. Though the Eck fistula by no means excludes the liver from function, and the degree of permanent increase of blood flow through the liver with the reversed Eck fistula is doubtful, genuine and unmistakable differences in the tendency to ketosis following these operations would be strongly suggestive.

In this study, no attempt was made to compare the fistula dogs rigidly with normal controls, because of the spontaneous differences in ketonuria between individual animals. The ketosis of the Eck dogs was merely compared in a general way with that shown by normal dogs in the preceding paper, in the belief that any significant difference should be perceptible in this way. Various other procedures were also attempted: (a) the reversed Eck fistula, supposedly increasing the venous blood flow through the liver; (b) anastomosis of one of the main divisions of the splenic artery with the splenic vein, or removal of the left kidney and anastomosis of its artery with the splenic vein, in order to send additional arterial blood through the liver; (c) the removal of as much liver tissue as possible, as a means of reducing liver function. Most of these numerous attempts came to nothing, because



of operative deaths, subsequent thrombosis of sutured vessels, or other accidents occurring before the experimental program could be completed. War conditions then halted the research, so that only a small group of observations with the Eck fistula and one with the reversed fistula are worth reporting.

Also, complete quantitative determinations of the blood lipoids of the fistula animals were included, but these results were among the mass of figures that were lost (see Series IV, Lipemia). It can be stated from memory that no special peculiarities, and in particular no increased tendency to lipemia, were found in the Eck fistula animals.

Dog F6-30 was a yellow and white male mongrel, aged 3 or 4 years and weighing 24 kg. in good nutritive condition. A reversed Eck fistula was established on Feb. 22, 1918. The dog recovered easily and had normal urine on bread diet.

April 1, catheterization was performed and fasting begun. April 5, catheterization was repeated and phlorization begun, with results shown in Table 1. In all these experiments, it is to be understood that urine was voided spontaneously and collected in the metabolism cages except when catheterization is mentioned.

After April 12, phlorizin was omitted and fasting was replaced by a diet of 1 kg. of beef lung. Ketosis cleared up promptly as usual, and did not return when 200 gm. of suet was added to the diet, though heavy glycosuria was still present. Lipemia was also absent.

*Remarks.*—The ketonuria in this dog with the reversed Eck fistula was greater than in any of the series with the direct Eck fistula. It was not greater, however, than may occasionally be exhibited by normal dogs, and the quantitative variations in this dog and in the Eck fistula dogs cannot be regarded as proof of a specific rôle of the liver, because of the spontaneous differences between dogs already mentioned. The clearing up of ketosis on protein diet, and its absence on protein-fat diet in the presence of heavy glycosuria, are identical with the conditions in normal dogs and reveal no exaggerated tendency to ketosis.

Dog F6-31 was a yellow male mongrel aged 2 years and weighing 19 kg. in a good nutritive state. An Eck fistula was established on May 2, 1918, and an oatmeal diet given subsequently. The dog recovered apparent health.

June 15, fasting begun. Subcutaneous injections of 1 gm. phlorizin in oil suspension were given on June 16, 18 and 20. Ketosis developed as shown in Table 2.

*Remarks.*—The Eck fistula seemed in no wise to inhibit ketosis in this dog. No intoxication symptoms were present in this instance. Autopsy a month later verified the permanency of the anastomosis and the complete closure of the portal vein above any communicating branches, as was also the case in the other animals which sooner or later came to autopsy.

Dog G7-24 was a female bird-dog mongrel aged 3 or 4 years, weighing 22 kg. in good nutritive condition. An Eck fistula was established by operation on June 12, 1918. The dog fasted continuously thereafter and showed normal urine and blood chemistry.

June 17, an injection of 1 gm. phlorizin in oil emulsion was given subcutaneously. Similar doses were given on June 18 and 19. On June 19 ketonuria was well marked and there was a faint nitroprusside reaction in the plasma. On that day at 11 P.M. there was a violent general convulsion. The chemical findings gave no explanation. There was the ordinary ketonuria, and the plasma sugar was 0.084 per cent., the plasma bicarbonate 66.0 volumes per cent., the nitroprusside reaction in the plasma faint, and only traces of acetone bodies found quantitatively. The blood was somewhat concentrated as judged by the corpuscle volume of 56.3 per cent.

June 20, the condition of the cage indicated that there had been no convulsions during the night. The dog was found limp and entirely unable to stand, but clearly conscious and attentive to surroundings, free from dyspnea or nausea, with normal pulse and temperature 38.2° C. The condition did not resemble coma. Moderate ketonuria continued. The plasma sugar was 0.105 per cent., the plasma bicarbonate 65.1 volumes per cent., total plasma acetone 28.4 mg. per 100 cc., and the corpuscle volume 46 per cent. Phlorizin was discontinued because of the danger of death. Numerous major and minor convulsions occurred in the course of the day, also several attacks of panting and crying as if from pain. A total of 2 liters of water was given by stomach tube as a precaution against desiccation. It was retained and caused free diuresis. No food or other treatment was given.

June 21, the clinical and chemical findings were similar. Convulsions continued with about the same frequency. After an interval of an hour without spasms, the rectal temperature was 39.7°, the pulse 204, the respiration 66 per minute. There was still no resemblance to coma, as consciousness was fully clear, and the dyspnea was limited to a shallow panting as if from warmth and was unlike the deep respiration of acidosis. An additional 500 cc. of water was given by stomach tube at 11 A.M. The condition being unchanged at 3 P.M., 500 cc. of 10 per cent. glucose solution was given by stomach tube, followed by 120 cc. intravenously. Several convulsions occurred during the process. At 11 P.M., an additional 500 cc. of 10 per cent. glucose solution was given by stomach tube, and the dog seemed better.

TABLE 1  
Dog F6-30

Date 1918	Urine						Blood Plasma						Remarks			
	Vol. cc.	Sugar gm.	Total N gm.	D/N ratio	Nitro- prusside	Acetone and Diacetic Acid mg.	B-oxy (as Acetone) gm.	Total Acetone gm.	Sugar mg. %	CO <sub>2</sub> Cap. Vol. %	Lipemia qual.	Nitro- prusside		Acetone and Diacetic Acid mg. %	B-oxy (as Acetone) mg. %	Total Acetone mg. %
Apr. 1	770	Neg.	9.36	.....	Neg.	.....	.....	.....	104	51.2	Neg.	Neg.	.....	.....	.....	Not fed.
2	158	"	3.42	.....	"	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	"
3	1450	"	6.30	.....	"	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	"
4	290	"	4.32	.....	"	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	"
5	238	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	"
6	665	39.1	.....	.....	Faint	Trace	0.088	0.088	114	49.6	Neg.	Neg.	.....	.....	.....	"
7	530	32.4	9.90	3.18	Neg.	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	"
8	700	25.9	11.62	2.22	Heavy	140	0.520	0.660	.....	.....	.....	.....	.....	.....	.....	"
9	820	33.8	15.48	2.28	"	250	0.990	1.240	.....	.....	.....	.....	.....	.....	.....	"
10	1150	63.2	16.68	3.79	"	820	1.680	2.500	90	50.0	Neg.	Faint	Trace	21.6	21.6	"
11	1300	59.2	17.16	3.45	"	820	2.730	3.500	.....	.....	.....	.....	.....	.....	.....	"
12	1280	53.0	15.31	3.46	"	560	1.950	2.510	78	48.8	Neg.	Faint	7.0	30.1	37.1	"
13	940	60.0	23.16	2.58	Mod.	Trace	0.600	0.600	.....	.....	.....	.....	.....	.....	.....	1 kg. lung.
14	1990	74.0	33.60	2.20	Neg.	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	1 "
15	2130	48.8	23.32	2.10	"	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	1 "
16	2230	40.0	21.62	1.85	"	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	1 "
17	1705	43.6	21.60	2.02	"	.....	.....	.....	97	52.3	Neg.	Neg.	.....	.....	.....	and 200 gm. suet.

TABLE 2  
Dog F6-31

Date 1918	Urine					Blood Plasma			Remarks
	Vol. cc.	Sugar gm.	Total N gm.	Nitro- prusside	Total Acetone gm.	Sugar mg. %	CO <sub>2</sub> Cap. Vol. %	Total Acetone mg. %	
June 16	.....	.....	.....	Neg.	.....	.....	.....	.....	1 gm. phlorizin.
17	740	52.27	6.22	Slight	.....	.....	.....	.....	
18	840	51.48	10.71	Mod.	.06	.....	.....	.....	1 "
19	1300	83.98	14.82	"	1.08	82	56.4	25.2	
20	1015	33.41	16.06	Heavy	.94	.....	.....	.....	1 "
21	1130	51.24	11.04	"	1.33	.....	.....	.....	
22	1250	45.63	17.29	Mod.	1.10	89	51.7	41.0	



June 22, the dog was stronger and had no convulsions. The plasma sugar was 0.086 per cent., the plasma bicarbonate 61.2 volumes per cent., and the corpuscle volume 56 per cent. Heavy glycosuria still continued, but urine and blood were free from acetone. The dog was given 500 cc. of 10 per cent. glucose solution by stomach tube.

June 23, the dog continued free from convulsions, and was able to sit up but not to stand. About 500 cc. of milk was given in divided quantities by stomach tube. Toward evening the dog drank water voluntarily but still refused food. One of the peculiar features throughout had been the refusal to eat or drink anything voluntarily, though the fluids given by tube were retained without any indication of nausea.

June 24, the dog could stand and began to take food, and the observations were terminated.

*Remarks.*—Hypothetically, the convulsions and accompanying condition could be explained as due to protein intoxication, not from the protein of food as usual, but from the increase of protein catabolism produced by phlorizin in the fasting animal. The cure by glucose would thus be clear. Owing to losses of urine and other disturbances introduced by the convulsions, no tabulation of quantitative results has been attempted. It seemed evident, however, that ketosis developed in the Eck-fistula animal in practically the same time and degree as in a normal dog.

Dog G7-59 was a female Collie aged about 4 years and weighing 21 kg. in good nutritive condition. An Eck fistula was established on July 5, 1918. The anastomosis seemed satisfactory and the surgical recovery was uneventful, but there was a general appearance of malaise and appetite was lacking. The animal was kept fasting continuously after the operation, and on July 8 received a subcutaneous injection of 1 gm. phlorizin in oil. Marked ketonuria began within 24 hours (Table 3). This, and also the high ammonia excretion of July 9 and 10, may possibly be due to the Collie breed.<sup>3</sup>

July 11, one violent convulsion occurred. Otherwise the dog appeared normal, moderately strong but slightly depressed. The chemical findings as shown in Table 3 gave no explanation.

July 12, the dog had repeated convulsions, and between them was limp, almost completely unconscious, and apparently dying, except for the strong regular pulse. At 3 P. M. the rectal temperature was 39.5° C., the pulse 150, and the respiration 84 per minute. This dyspnea came on only in occasional attacks and was merely a panting, unlike the breathing of acidosis coma. The external jugular was exposed without any sign of feeling on the part of the dog. A sample of blood from it gave the analyses shown in the table. Immediately thereafter, 100 cc. of 20 per cent. glucose solution was injected into the vein, with prompt and striking benefit. The dog became partially conscious and able to sit up by being braced against the side of the cage. Half an hour later, 200 cc. of 10 per cent. glucose

TABLE 3  
Dog G7-59

Date 1918	Urine					Blood Plasma				Remarks	
	Vol. cc.	Sugar gm.	Total N gm.	NH <sub>3</sub> -N gm.	Nitro- prusside	Total Acetone gm.	Sugar mg. %	CO <sub>2</sub> Cap. Vol. %	Corp. Vol. %		To Acetone mg. %
July 8	600	11.76	7.68	0.30	Faint	.....	.....	.....	.....	.....	1 gm. phlorzin.
9	1570	29.60	12.48	2.24	Mod.	0.12	.....	.....	.....	.....	1 "
10	1400	22.12	8.96	2.10	"	1.84	.....	.....	.....	.....	"
11	840	20.88	8.10	0.43	"	0.95	.....	60.5	47.0	26	
12	146	9.52	1.18	0.07	Heavy	0.31	73	51.9	49.6	39	

TABLE 4  
Dog F6-67

Date 1918	Urine					Blood Plasma			Remarks
	Vol. cc.	Sugar gm.	Total N gm.	D/N ratio	Nitro- prusside	Sugar mg. %	CO <sub>2</sub> Cap. Vol. %		
May 27	645	10.6	2.87	3.70	Neg.	.....	.....		1 gm. phlorizin.
28	1340	20.3	10.78	1.88	"	.....	.....		1 "
30	720	23.5	7.04	3.34	V. f.	.....	.....		1 "
29	900	34.7	9.18	3.78	"	.....	.....		1 "
31	720	50.0	8.24	6.06	"	92	51.9		1 "
June 1	810	24.4	8.92	2.73	Slight	.....	.....		1 "
2	855	Heavy	11.34	.....	Neg.	.....	.....		1 "
3	640	14.8	5.80	2.05	"	84	52.3		1 "

solution was injected intravenously, and 300 cc. water was given by stomach tube and retained, but there was no further benefit. At 4:45 P.M., 250 cc. of 10 per cent. glucose solution was given by stomach tube. At 5:45, there was an attack of intense dyspnea, with panting respirations 144 per minute, not helped by the giving of 500 cc. of water by stomach tube. The dog was left for a brief time, and was found freshly dead at 6:15. The body was noticeably hot, and the rectal temperature was 42.3° C.

Autopsy was negative except for intense congestion of the viscera, brain and meninges. Examination verified the free communication between the cava and portal vein, and the complete ligature of the latter higher up, above any discoverable anastomoses.

*Remarks.*—Though the intoxication cut short the experiment, it seemed apparent that the Eck fistula did not alter the tendency to ketosis.

Dog F6-67, a male mongrel, aged 4 or 5 years and weighing 23 kg. in a good nutritive state, had an Eck fistula established by operation on May 22, 1918. Continuous fasting was imposed thereafter. Three subcutaneous injections of phlorizin suspended in oil were given, on May 27 and 30 and June 2, as shown in Table 4.

There were no intoxication symptoms, and no ketosis sufficient for positive quantitative determination in either urine or blood. The nitroprusside reaction was negative in the plasma, and was barely perceptible in the urine for the few days up to June 1 and thereafter absent. The dog showed merely a progressive decline of strength, so that after June 3 it was considered necessary to break off the experiment and begin bread diet.

The appetite and strength were somewhat subnormal, but the general condition seemed fair until the dog was unexpectedly found dead on June 29. Autopsy showed a surprisingly extensive blood-clot, which had evidently started at an angle of the venous anastomosis. It did not completely plug the anastomosis, and extended only a couple of centimeters into the portal vein or toward the heart in the cava. But it extended caudad in the cava clear to the junction of the two iliacs, occupying most of the lumen of the vein but yet not occluding it entirely. It also extended into some of the tributary veins, especially into both renal veins. The renal veins were completely plugged with the clot, but as some portions of this were fresh, it is probable that some circulation continued till near the time of death. The portal domain was not seriously congested and most of the viscera were normal grossly and microscopically. The kidneys showed intense stasis but no necrosis. It is probable that death occurred from uremia, but as no such condition had been suspected there were no tests to confirm this interpretation.

*Remarks.*—This dog seemed highly exceptional in its failure to develop any appreciable ketosis. Therefore, though in other animals the Eck fistula showed no influence in preventing ketosis, there is a suggestion that some sort of abnormal condition is possible which can make the organism relatively resistant to ketosis.



### *Conclusion*

The susceptibility of dogs to ketosis with fasting and phlorizin was not obviously altered by the Eck fistula. The doctrine of the exclusive or predominant formation of acetone bodies in the liver, which seems contrary to the best modern conceptions of metabolism, is therefore not supported by these experiments.

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## AUSCULTATORY ESTIMATION OF THE BLOOD PRESSURE OF DOGS

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There has long been general recognition of the need for a simple and reasonably accurate method of making repeated blood pressure estimations without anesthesia or other disturbance in laboratory animals, especially dogs. When a series of experimental studies of nephritis and hypertension was contemplated in this Institute, this technical obstacle was inevitably one of the first problems that engaged our attention. After two years of unsatisfactory trials of more complex methods, we have learned within the past year that the systolic and diastolic pressures of dogs can be taken by auscultation in nearly the same way and apparently with a similar degree of accuracy as in man. This paper will give a brief description of this method and its results. The following is a partial review of the literature concerning (a) methods used heretofore, and (b) the blood pressure values thus obtained in normal dogs.

### *Methods of Blood Pressure Determination*

*Cannulation.*—Pawlow<sup>1</sup> inserted a cannula into the small artery on the inner aspect of the knee joint of dogs. He asserted that this small operation could be performed within 2 or 3 minutes, without anesthesia and without pain. Nevertheless, this early suggestion has not been followed, but the vast majority of laboratory observations of blood pressure have been made by cannulation of the largest arteries (carotid and femoral) under anesthesia.

A review of many of these is unnecessary in the present connection. Pässler and Heineke,<sup>2</sup> however, actually made use of such a method for their studies of the influence of partial nephrectomy upon blood pressure, one femoral artery being used for taking the pressure before operation, and the other femoral artery similarly used some time after operation. They avoided the use of the carotids for successive blood pressure determinations, on the basis of observations by Tangl and Zuntz.<sup>3</sup>

Brooks<sup>4</sup> suggested three ways of taking the carotid pressure, after first dissecting the artery free under ether and anchoring it close under the skin. A period generally of 24 hours (sometimes half a day, or 2 or 3 days) was allowed for recovery from the anesthetic. Then the pressure

was taken painlessly by (a) withdrawing a paraffin-soaked cotton plug from the side-arm of a three-way cannula which had been inserted in the artery at the time of operation, (b) inserting a cannula in the ordinary manner, neither general nor local anesthesia being required because of the previous isolation of the artery; or (c) plunging a special trocar cannula into the artery, withdrawing its obturator and taking the pressure, then withdrawing the cannula and compressing and massaging the artery for 5 minutes to stop bleeding. The last method was specially recommended as a means for obtaining usually several readings at successive intervals before the artery finally clotted shut.

P. Trendelenburg and Fleischhauer<sup>5</sup> likewise prepared the carotids of rabbits under anesthesia and made pressure readings after their recovery.

Turner, Marshall and Lamson<sup>6</sup> found pressure determinations by cannulation of the femoral artery unsatisfactory under ether anesthesia, and therefore performed the operation under local cocain and made the readings with a slightly modified manometer.

*Non-cannulating or "bloodless" methods.*—Gaertner<sup>7</sup> applied a tonometer to the tails of dogs and claimed to obtain reliable blood pressure readings, though admitting that the color changes of the skin here were rather difficult to distinguish. Trendelenburg<sup>8</sup> applied the same method to the legs of cats, using the changes in the normal pink color of the paws for determining the blood pressure.

Erlanger,<sup>9</sup> using dogs, dissected the trachea free, and then applied the band of a sphygmomanometer beneath the trachea around all the other tissues of the neck. He also converted the "cone" of a dog's thigh into a "cylinder" by excision of some of the muscles, in order that a sphygmomanometer band might thus be applied without slipping.

Van Leersum<sup>10</sup> made two longitudinal incisions in the skin of a rabbit's neck, 3 or 4 cm. long by 1 cm. wide, and used the skin-flap thus formed for enveloping the carotid artery. After healing, the artery thus isolated could be encircled by a tiny manometric cuff, and estimations of the systolic pressure made by finger palpation. Diastolic observations or graphic records were not obtainable.

Janeway<sup>11</sup> applied the cuff of a sphygmomanometer to the lower part of the fore leg of large dogs, and estimated the systolic pressure by palpation of the small artery in the ball of the foot.

Kolls<sup>12</sup> devised a special cuff, consisting of a rounded and tapering aluminum plate to fit the external surface of the thighs of dogs, and a flap of moleskin cloth to go across the flat internal surface. The other feature of the apparatus is a special sphygmomanometer delicate enough to record on a smoked drum the pulsations transmitted from the artery. "The criteria employed for the estimation of systolic and diastolic pressures were those customarily used, namely, the spreading of the limbs of the pulse tracing for the first mentioned and the sudden decrease in amplitude for the last. In six such determinations the maximum differences between the direct and indirect determinations did not exceed 5 mm." There is no doubt that the work of Kolls marks an important advance and affords the



method of choice where graphic records of the indirect blood pressure are desired.

Tigerstedt<sup>13</sup> states that animal experiments, in which the blood pressure was taken in one femoral artery by direct connection with a mercury manometer and in the other by the Riva-Rocci apparatus, have shown almost perfect agreement between the two methods. He gives no reference to the literature. Such a comparison between the direct and the auscultatory method was made by Warfield,<sup>14</sup> but the femoral artery was exposed for this purpose and surrounded by a small water-chamber for the transmission of the sound. This author cites the literature of other comparisons of this kind, by which the validity of the auscultatory principle was established; but there seems to be no account of any practicable auscultatory method for routine repetition in animals, and certainly no such method has come into general knowledge or use.

#### *Reported Blood Pressures of Dogs*

Pawlow<sup>1</sup> obtained systolic pressures of 128 to 150 mm. of mercury in unanesthetized dogs, indicating a very creditable accuracy in these early observations.

The records of Turner, Marshall and Lamson<sup>6</sup> show such an abnormal initial pressure as 208 mm., which is evidence that their dogs, though free from pain, were not free from excitement. Influences such as emotion, exertion, etc., are understood to be important in animals, just as in man. Tangl and Zuntz<sup>3</sup> found only slight effects from slight activity, but with heavy exercise systolic pressures as high as 234 and 242 mm. Hg.

Special attention must be given to the findings of skilled workers, who have used maximum and minimum valves or other devices for obtaining exact figures such as are not afforded by the simple mercury manometer. Thus Erlanger<sup>9</sup> obtained intra-arterial (*profunda femoris*) pressures as follows: maximum (systolic) generally 152 to 170 (sometimes as low as 100 to 120); minimum (diastolic) generally 104 to 110 (sometimes as low as 75 to 90). The findings of Dawson<sup>15</sup> were 152 to 188 systolic, 92 to 123 diastolic. Wiggers<sup>16</sup> in his Table II shows systolic carotid pressures ranging from 126 to 250 mm. Howell<sup>17</sup> makes the generalization that the systolic pressure in the dog's aorta under ordinary experimental conditions is "as much as 168 mm., while the diastolic pressure is only 100 mm." He also accepts the figures of Volkmann (*Die Haemodynamik*, 1850) for the dog's blood pressure as 172 systolic and 104 diastolic.

It must be recognized that all the figures quoted in this last paragraph represent the abnormal state of anesthesia, and therefore are not a normal standard. Turning from the direct to the indirect methods, the tonometric findings must be rejected as unreliable, or at least too low to represent the pressure in the large arteries. Janeway's method, according to his own statement, was "rough," yielded only relative not absolute values, and gave errors on the low side. Thus his observations of average systolic pressures between 91 and 119 mm. (highest single reading 130, lowest 80 mm.) must be discarded as too low.

Kolls and Cash<sup>18</sup> reported the results of 285 observations on 12 normal

dogs with their new method. The average systolic pressure was 165 and the average diastolic 61 mm. Hg. Their large table shows considerable individual differences, the highest systolic pressure being 200 mm. The greatest variations were found in the diastolic, for which their highest reading was 104 and their lowest 28 mm. They note that the pressure may be changed by such small disturbances as a noise in the laboratory, or clipping the hair of a leg. It will be observed that their systolic pressures correspond approximately to those in the literature, but they emphasize the lower diastolic figures as probably representing normal conditions more correctly than the high values obtained under anesthesia.

### *Development of Method*

The purpose was to find a simple auscultatory method of taking dogs' blood pressures, and with the aid of successive assistants this attempt was carried on intermittently without success for two years. The chief initial idea was that dogs differ from men mainly in the smaller size of the arteries available for the purpose, and that readings should be obtainable by the use of sufficiently delicate apparatus. An expert in the manufacture of electrical and other instruments of precision was employed for some time to make various microphones, by which it was hoped the significant sounds could be detected when a cuff was applied to either the front or the hind leg, but none of these contrivances proved practicable. Attempted modifications of the plethysmographic principle, by immersing a limb in a closed vessel of liquid, likewise yielded nothing. Surgical preparation of the carotid according to van Leersum, or of the thigh according to Erlanger, proved to be difficult, time-consuming, mutilating, and unsatisfactory in results. Returning to the simplest possible devices, namely the ordinary cuff and stethoscope as used in human observations, Drs. E. F. F. Copp and Robert Mark proved that by slight modifications it was possible to obtain clear auscultatory readings. The writer wishes to assume responsibility for the guidance of the investigation and for any theoretical or practical errors, but most of the final details should be credited to the ingenuity of the two assistants mentioned.

### *Apparatus*

The only essential parts are an ordinary sphygmomanometer (we used a Baumanometer), a cuff of the size used for infants, and a stethoscope with a special bell-piece. This bell is of the phonendoscope type, small and flat (about 2 cm. in diameter and

0.75 cm. thick), with a celluloid diaphragm. A small metal ring is soldered to each side of it, for attaching tapes. The blood pressure can be taken in any position of the animal, but the most convenient routine has been to have the dog standing up. At first an assistant steadied the animal, but a wooden frame or stand was found to be labor-saving and also more satisfactory. The dog stands between the uprights, and two or three broad canvas bands are buckled across under the body to prevent the usual tendency to sit or lie down. A small strap to hold the tail quiet is an additional help. The details of the entire apparatus will probably be clear from inspection of figures, 1 and 2, which were kindly sketched by Dr. Arnold Zimmermann.

### *Procedure*

The dog is placed in the frame and the straps adjusted for support. The lower part of the hind leg, which has previously been cleanly shaved, is palpated for the course of the femoral artery, which ordinarily can be easily traced to the inner and anterior aspect of the limb above the ankle joint. The stethoscope bell is applied to the artery and held in place by tying the two tapes, not tightly enough to cause any appreciable compression. The cuff is then wound about this part of the leg, so that its lower portion covers the stethoscope bell. In the beginning of our work the cuff was applied first, and then the bell, without tapes, was slipped inside its lower border, but experience showed the preliminary securing of the bell with tapes to be an advantage. The essential point is that the bell lies accurately on the artery inside the lower border of the cuff. Readings are not obtainable by attempting to listen with the bell at some point outside and below the cuff, as done with human subjects.

The observations are then made precisely as in human practice. The cuff is inflated until the pulse is cut off, and the mercury allowed to fall slowly until the first regular pulse sounds are heard. This level is recorded as the systolic pressure. With further escape of air, a point is reached where the sounds suddenly and definitely become fainter. We have assumed this level as representing the diastolic pressure. Sometimes the sounds persist to levels as low as 25 to 35 mm. before they completely disappear. We have chosen the higher level mentioned because of clinical custom and the literature underlying the auscultatory



method (cf. Warfield<sup>14</sup>), and also because it is less variable in different animals and also is more precisely ascertainable, as the exact point of the fading out of the last faint sounds is sometimes uncertain.

The sounds obtained with this method are not as loud but are practically as sharp and plain as in human subjects. A practiced operator can thus take pressures as easily and quickly in dogs as in men. Anybody accustomed to the clinical procedure immediately recognizes the similarity of the sounds in dogs and after a few trials can make correct readings. Practice with the adjustment of the bell and cuff and other details increases accuracy, but in general different observers can check their results as closely as in clinical work.

#### *Precautions and Limitations.*

It is presumed that shaving of the leg has been performed at some previous time, and that the dog is accustomed to handling. The effects of excitement are well known and easily demonstrable. Holding up a cat in front of one of our nervous dog would increase the systolic pressure by 40 to 60 mm. within less than a minute. All dogs have quickly learned to stand quietly in the frame and to undergo all the manipulations without disturbance, so that an operator can rapidly make a series of observations on trained dogs without the need of an assistant.

In general, the larger the dogs, the better for blood pressure determinations. We seldom try to use dogs of less than 10 kg. for this purpose. An occasional large animal is unsuitable. Some dogs have such irregularity of the heart action, not only in rhythm but also in force, that satisfactory blood pressure estimations are impracticable regardless of the method. Also, either the distribution or the structure of the arteries seems to be inconsistent, so that even large dogs sometimes fail to furnish clear pulse sounds. On the other hand, some small dogs give unusually distinct sounds and are entirely fit for such experiments. As a rule dogs with long slim legs are more suitable than those with short thick ones, and animals of the collie type have been among the best.

Blood pressure readings have been obtained by this method with some difficulty and uncertainty in sheep, but attempts with goats have thus far been unsuccessful.

TABLE I

Dog No. 110			Dog "L"				
Date	Systolic Pressure	Diastolic Pressure	Date	Systolic Pressure	Diastolic Pressure	Systolic Pressure	Diastolic Pressure
Aug. 1	159	56		Taken by A		Taken by B	
" 1	162	58					
" 1	160	60	Aug. 1	162	88	166	90
" 2	156	60		" 2	154	54	158
" 2	142	50		" 3	152	70	152
" 3	145	70		" 4	142	84	152
" 3	138	72		" 9	158	65	140
" 4	152	75		" 10	164	76	148
" 4	158	78		" 13	135	68	134
" 5	130	68					
" 5	126	64					
Sept. 1	146	60	Average systotic reading for A: 152.				
" 1	140	58	Average systotic reading for B: 150.				
" 2	154	80	Average diastolic reading for A: 72.				
" 2	164	78	Average diastolic reading for B: 73.				
" 3	160	82					
" 3	176	84					
" 4	152	70					
" 4	154	68					

### *Results in Normal Dogs*

We have made considerably over a thousand pressure readings in more than 60 normal dogs. Table 1 shows typical results on two animals, and also a typical comparison of results of two observers who recorded their findings before comparing them.

Table 2 summarizes the findings in 31 well trained quiet dogs upon which regular blood pressure observations were made for periods ranging from 6 days to 4 months.

It is noticeable that not only is the method similar to that used in man, but also it shows a closer correspondence than heretofore accepted between dog and man as respects systolic, diastolic and pulse pressures. There are individual differences, somewhat as in human subjects, but no regular distinctions could be made out between adult dogs in ordinary nutritive states on the basis of age, sex, or weight. The upper range of the systolic pressure seems to be higher in the dog than in man. We consider it doubtful if rare maximum readings, such as 176 mm., can be considered as a strictly normal blood pressure in any dog, at least by this

method. Possibly 164 is a more conservative figure for the normal maximum, but in the young human adult 160 mm. is to be regarded as distinctly abnormal. The diastolic readings compare fairly well with those in man. They are almost never as high as those obtained under anesthesia. On the other hand our average (79 mm.) is higher than that obtained by Kolls and Cash with their method (61 mm.), and ours could not be reduced to their level unless by adopting the point at which pulse sounds completely disappear. The pulse pressure by our method is generally greater than recorded for etherized dogs, but is very much less than that obtained by Kolls and Cash, on account of both the lower average systolic and the higher average diastolic by our method. It corresponds more closely with the usual pulse pressure in man.

TABLE II

Dog No.	Average Systolic Pressure	Average Diastolic Pressure	Dog No.	Average Systolic Pressure	Average Diastolic Pressure
0	140	78	90	128	82
46	159	74	162	144	84
115	162	84	163	123	59
168	138	90	164	139	81
159	124	66	151	110	67
148	125	67	170	131	74
J	132	80	172	136	71
110	148	65	230	135	90
L	151	72	223	120	80
161	134	77	241	165	75
160	148	77	258	134	86
158	147	84	263	134	82
153	156	99	240	130	76
152	159	79	281	160	110
165	138	70	282	130	70
232	130	90			

General averages:

Systolic pressure 139

Diastolic pressure 79

Pulse pressure 60

### *Control Observations*

Control experiments were performed only with an ordinary mercury manometer, without maximum-minimum valves or other devices for greater exactness. Criticisms are therefore possible



that mean rather than systolic pressures were obtained, and that there is no experimental support for our assumption of the level of the diastolic pressures. We did not wish to be drawn aside into the problem of rigidly accurate blood pressure determinations, and felt able to rest to some extent on the general evidence supporting the use of the auscultatory method in man. We were chiefly interested, like Janeway, in a simple method for obtaining at least comparative values, and if changes of blood pressure shown by our method corresponded reasonably well with those shown by a mercury manometer connected with an artery, we felt that this method would serve the purpose in view.

A large quiet female dog was selected, and the auscultatory blood pressure readings at 11 A. M. were 134 systolic, 86 diastolic. The dog was then etherized, and during the anesthesia higher but very irregular values were obtained for both the systolic and diastolic. The left carotid artery and jugular vein were dissected free, the skin closed with temporary sutures, and the dog allowed to recover, the whole procedure occupying only a few minutes. At 3 P. M. the animal appeared fully normal, and auscultatory blood pressure observations were made and recorded independently by three persons, as follows:

A obtained systolic 138, diastolic 75.

B obtained systolic 143, diastolic 76.

C obtained systolic 140, diastolic 74.

It proved necessary to etherize again in order to keep the animal sufficiently quiet for the simultaneous observation of the pressure by direct and indirect methods. The carotid was cannulated, and one pair of workers recorded the maximum differences between the two limbs of the mercury manometer, while another pair made simultaneous records of the auscultatory pressure. The comparison between the two records is shown in Table 3.

Occasional records of the minimum differences between the mercury limbs in connection with the carotid averaged 108 mm., while simultaneous auscultatory readings of the diastolic pressure averaged 104 mm.

At 4:15 P. M., a slow injection of 1/25,000 adrenalin solution into the exposed jugular vein was begun. The rise of pressure, and its fall after discontinuance of the injection, are shown in the comparative readings.

Not only the faults of the simple mercury manometer, but also the differences between the carotid and a branch of the femoral artery, make such comparisons merely approximate. The parallelism of the observed changes, however, supported the belief in the practical usefulness of the auscultatory method.

#### *Abnormalities of Pressure*

Abnormally low systolic and diastolic pressures have been found in states of extreme weakness in dogs, the same as in human patients. Consistently high values, not temporary effects of

TABLE III

Time P. M.	Direct Pressure	Indirect Pressure	Remarks
	...	140	Before anesthesia. Etherization at 3:17 p.m.
3:30	174	...	
3:35	177	...	
3:40	179	176	
3:45	178	178	
3:50	176	172	
3:51	177	...	
3:52	175	168	
3:55	164	156	
3:56	169	158	
3:58	175	164	
3:59	177	170	
4:00	178	176	
4:01	180	180	
4:02	160	...	
4:04	160	154	
4:05	167	160	
4:06	176	170	
4:08	166	...	
4:09	164	162	
4:10	162	165	
4:15	174	168	Adrenalin injection into jugular.
4:16	200	200	
4:20	202	196	Injection stopped.
4:21	210	202	
4:25	159	152	

excitement or exertion, have been found only as an accompaniment of kidney disease. A collie with spontaneous nephritis showed pressures of 220 systolic, 122 diastolic. The study of this animal is still in progress. As far as we are aware, this is the first record proving that dogs are subject to spontaneous hypertension, like man.

At the meeting of the American Society for Experimental Pathology in Toronto on Dec. 28, 1922, a paper by the present writer on "Experimental Hypertension" was read by title. This paper was in part a confirmation of the reports by Pässler and Heineke and by Janeway, that after removal of one kidney and half or slightly more than half of the other, dogs are liable to develop a moderate degree of arterial hypertension. This paper has not been published because the hypertension was demonstrated only by a few direct tests; i.e., the two carotids and two femorals served for four tests by a method like that of Brooks,

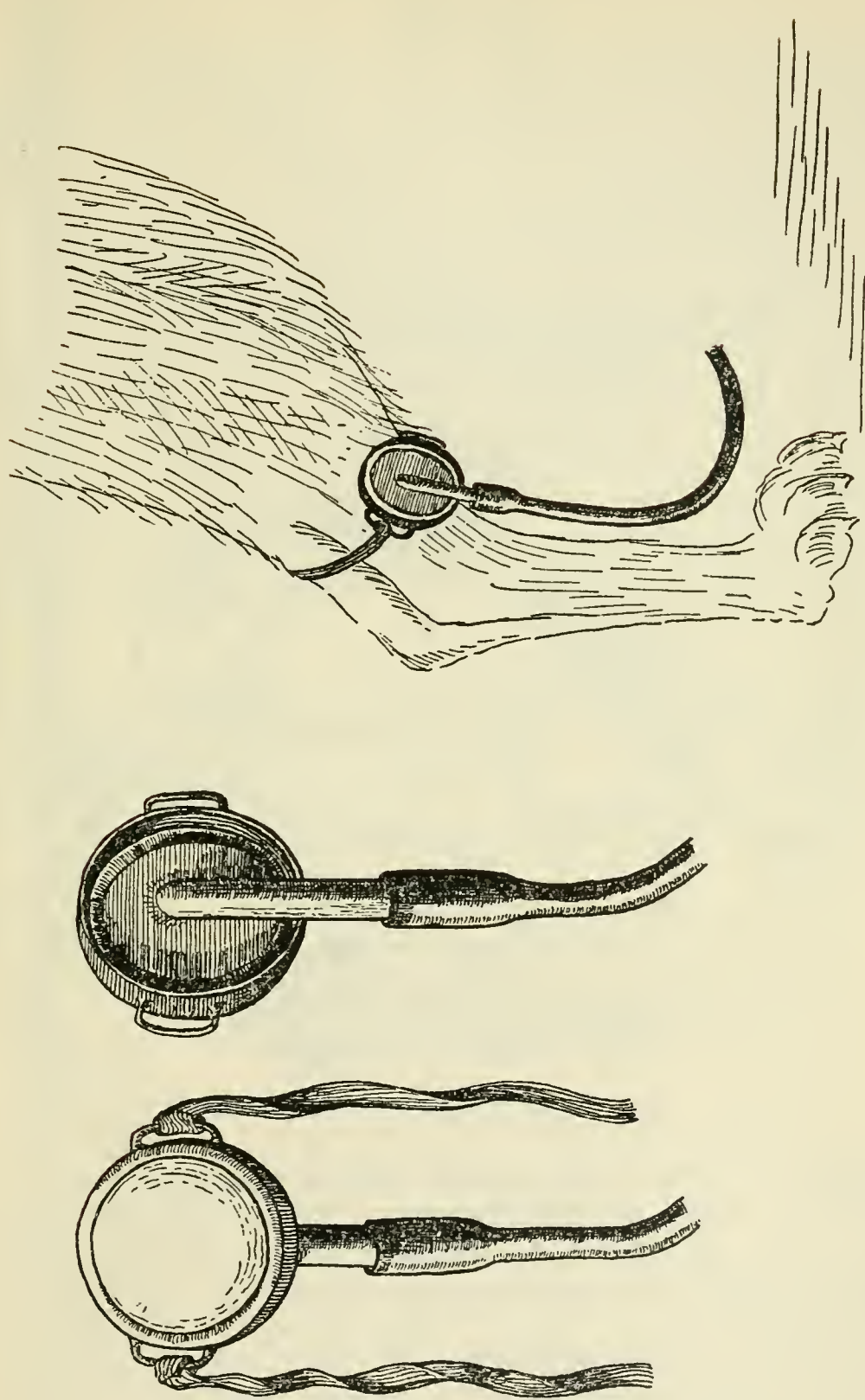


Fig. 1. Stethoscope, and its application to dog's leg.



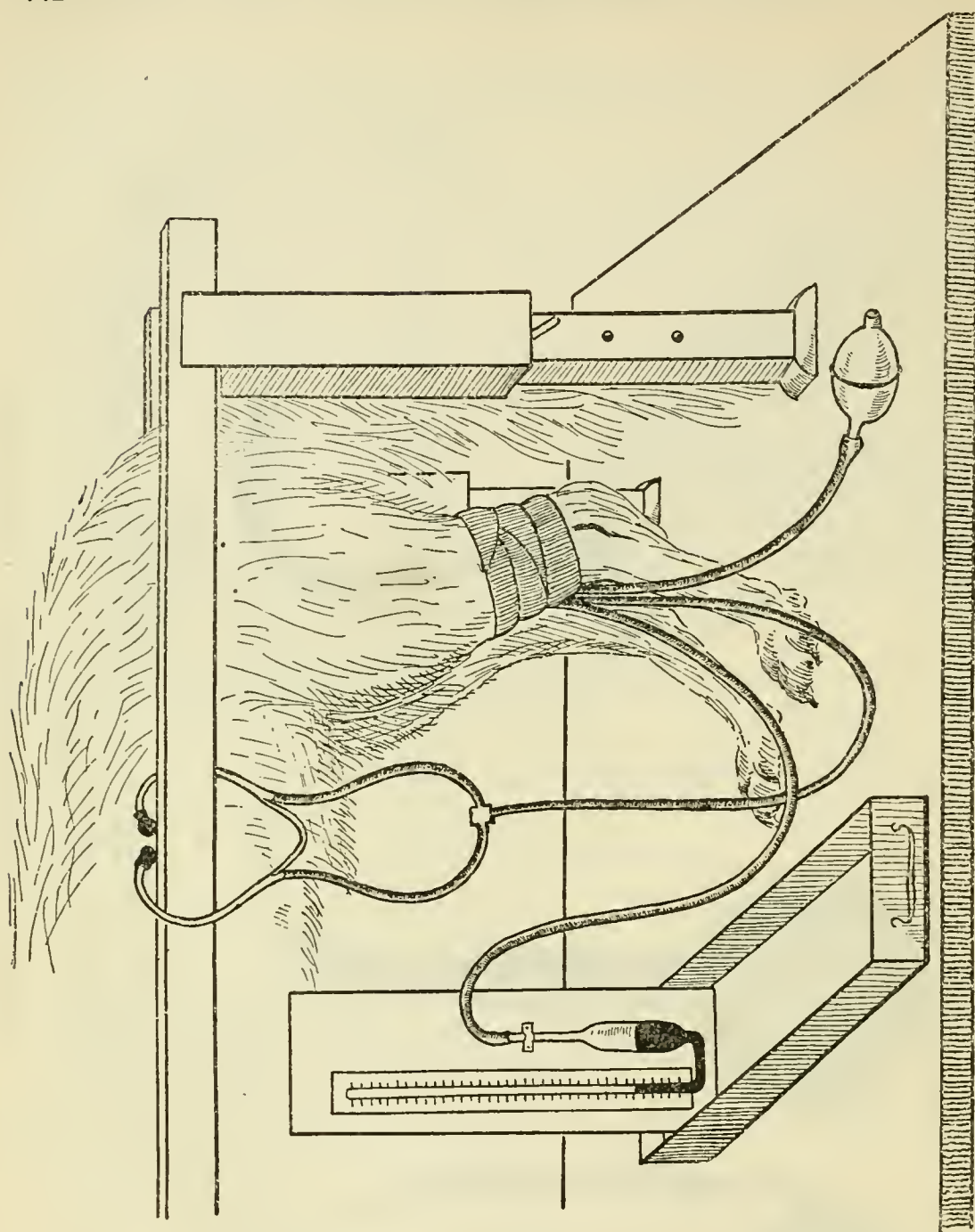


Fig. 2. Dog in stand, with manometer cuff applied over stethoscope.

exposing the vessels under general or local anesthesia and then cannulating to take the pressure after full recovery some hours later. It was suspected that criticisms would be directed at this method of testing. These results have now been confirmed by a larger number of auscultatory observations. The elevation of pressure with certain diets, and the fall with cachexia, will be described in later publications. The changes produced by other means than partial nephrectomy are also being studied further by the aid of the auscultatory method.

### Summary

By a slight modification of the clinical auscultatory method it has been found possible to make estimations of the systolic and diastolic blood pressures of suitable dogs. These estimations show a satisfactory parallelism with the readings of a mercury manometer connected directly with the carotid artery.

The variations in pressure of a series of normal dogs are shown in tables. The averages for this series were 139 mm. systolic pressure, 79 mm. diastolic pressure, and 60 mm. pulse pressure. If these values are correct in an absolute sense, they tend to bring the blood pressure of the quiet unanesthetized dog into closer correspondence with that of man.

The new method has proved suitable for demonstrating hypertension occurring spontaneously or produced experimentally in dogs. One marked instance of spontaneous nephritis and hypertension is mentioned, with 220 mm. systolic and 122 mm. diastolic pressure.

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# THE RESTORATION OF HYDROPICALLY DEGENERATED CELLS OF THE PANCREATIC ISLANDS IN DOGS UNDER INSULIN TREATMENT

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In his study of hydropic degeneration, Allen<sup>1</sup> made attempts to learn whether this change is reversible, i.e., whether the degenerated cells can recover normal size and granulation, particularly after they have reached the stage of maximal vacuolation. The results were indecisive, because of the lack of a method for checking the severest diabetes. "If pancreatic tissue is removed at the desired stage of maximal vacuolation, either the diabetes is already hopeless or the slight trauma of the operation makes it so, for it is impossible to stop the glycosuria thereafter, and with continued symptoms rapid destruction of islands occurs." From other observations, the conclusion was considered probable that the process is reversible, and that the vacuolated cells can recover if the cell membrane or the nucleus is not yet destroyed.

Insulin now furnishes a quick and sure means of arresting diabetic symptoms and relieving the strain upon the island function. It thus becomes possible to decide experimentally the question of the reversibility of hydropic degeneration.

## *Method*

The plan of experiment was the same as used by Allen. Diabetes was produced in dogs by removal of the pancreas except for a remnant about the main duct, amounting to 1/9th to 1/14th of the whole gland. This diabetes was then made severe by a period of overfeeding and constant heavy glycosuria. The purpose was to reach a stage at which practically all the beta cells of all the islands were completely vacuolated, but before extensive loss of cells or islands had yet occurred. The length of the period for this purpose was chosen according to the data on time relations given by Allen.<sup>1</sup> A second operation was then performed to obtain a small piece of the pancreas remnant for microscopic study. Insulin was given immediately at the close of this operation, and the treatment was continued so as to keep the animal continuously free from both glycosuria and hyperglycemia as strictly as feasible. At a subsequent operation or autopsy,

the remaining pancreatic tissue was examined for evidences of recovery in the islands. The time requirements for such recovery were entirely unknown and had to be learned experimentally.

#### *Preparation of Sections*

Only one fixative was used for these specimens, namely Zenker's fluid to which one per cent. acetic acid was added at the time of use. Fixing was continued for from eighteen to twenty-four hours and the tissues then washed in water until all traces of dichromate had been removed. Tissues were then passed through the alcohols each for twenty-four hours, dehydrated in absolute alcohol, cleared in toluol and imbedded in paraffin. Alcoholic iodine was added to the eighty-two per cent. alcohol a few drops at a time until no further bleaching occurred.

Sections cut three and four microns in thickness were stained in methylene blue-eosin stains. Distinct staining qualities were found to be gained in the use of very dilute solutions of each stain, the most favorable being about 0.1 per cent. of stain in distilled water. Staining was quite rapid, usually requiring but three to five minutes in each solution. Careful differentiation was carried out in ninety-five per cent. alcohol, checked in absolute alcohol, then cleared in toluol and mounted in neutral balsam.

#### *Results*

The most informative results were obtained in six animals, and are summarized in table 1.

Dog No. 162 was kept heavily glycosuric for 31 days after removal of eight-ninths of the pancreas. An ideal stage of degeneration was found in the 2 gm. of pancreatic tissue then removed, namely islands apparently normal in number and size but with maximal vacuolation of all their beta cells. After nine days of freedom from diabetic symptoms under insulin treatment, another pancreatic specimen was obtained which showed similar number and size of islands, with only slight vacuolation remaining in a minority of the cells. After fifteen days of further control of the diabetes with insulin, the islands found at autopsy were indistinguishable from those of a normal animal.

In dog No. 195, diabetes was only temporary after removal of nine-tenths of the pancreas, owing to hypertrophy of the remnant. Severe diabetes came on following the removal of an additional 1 gm. of tissue, and a pancreatic specimen obtained 20 days later showed advanced vacuolation of islands. During the following fifteen days under strict insulin treatment, the islands recovered completely normal appearance. After this operation, diabetes uncontrolled for four days resulted in a barely perceptible return of hydropic degeneration.

TABLE I

Dog No.	Weight (lb.)	Portion of Pancreas Removed	Interval Since Operation (days)	Diet	Insulin (units daily)	Clinical Progress	Pathology of Pancreas
162	36.0 21.5 .....	8/9 2 gm. .....	31 9 15	Bread and meat..... 400 gm. lean meat t.i.d. " " " "	..... 36 to 74 74 to 46	Constant heavy glycosuria..... Nearly perfect control..... " " " "	Islands in advanced degeneration. Only slight vacuolation. Islands normal. ..... ..... Advanced vacuolation. Islands normal. Slight beginning of island degeneration.
195	27.5 31.0 28.5 31.5	9/10 1.0 gm. 0.2 gm. Autopsy	35 20 15 4	Bread and meat..... " " " " 250 gm. lean meat t.i.d. " " " "	..... ..... 45 to 38 38 to 20	Short period of glycosuria..... Persistent glycosuria..... Diabetes well controlled..... Diabetes not controlled.....	..... ..... Advanced vacuolation. Islands normal. Slight beginning of island degeneration.
227	24.5	9/10	40	Mixed diet.....	.....	No glycosuria for 20 days; thereafter constant heavy sugar.....	Islands somewhat reduced in size and number. Advanced vacuolation. Cell cords and minor ducts thinned of cytoplasm.
	27.5	0.2 gm.	14	250 gm. lean meat t.i.d.	46 to 40	Well controlled.....	One cell in series of 50 sections appears vacuolated. All others normal.
226	25.5 21.5	12/13 0.2 gm.	28 12	Bread and meat..... 250 gm. lean meat t.i.d.	..... 32	Heavy glycosuria..... Well controlled.....	One or two groups of a few beta cells found. Cells cords and minor ducts extensively thinned of cytoplasm. No islands found; acinar tissue, cell cords and ducts normal.
148	38.0 32.5	10/11 .....	28 41	Bread and meat..... Unweighed bread & meat	..... 25 to 325	Heavy glycosuria..... Fairly well controlled.....	Islands large, numerous, extensively vacuo- lated. Islands normal in number, size and appearance.
164	24.0 ..... 26.0	12/13 0.5 gm. 0.2 gm.	31 38 55	Unweighed bread & meat..... " " " " " " " "	..... ..... 30	Heavy for few days only..... Constant heavy glycosuria..... Not well controlled.....	Islands large and numerous. Very few cells vacuolated. Islands slightly smaller, numerous. Moderate to advanced vacuolation. Slight vacuolation. Number of islands as before.



In dog No. 227, the second operation was performed 40 days after the first. Such a period of severe diabetes would ordinarily result in extensive loss of islands, but as glycosuria was present only in the latter half of this time, the islands were found present, though vacuolated. Fourteen days of control of the diabetes with insulin then resulted in practically complete restoration of the islands, only one cell being found which still appeared vacuolated.

Dog No. 226 had more severe diabetes than the preceding animals, by reason of the removal of twelve-thirteenths of the pancreas. Accordingly the beta cells were found to be practically completely lost after twenty-eight days of intense symptoms. The subsequent period of control of symptoms by insulin brought no sign of new formation of islands.

Dog No. 148 had a remnant of one-eleventh of the pancreas. After 28 days of constant glycosuria, the islands were apparently normal in number and size but maximally vacuolated. After 41 days of insulin treatment, the islands appeared unchanged in number and size, and their cells were normal in form and granulation.

Dog No. 164 underwent removal of twelve-thirteenths of the pancreas, but the diet was restricted so that glycosuria was absent for over three weeks. Accordingly the pancreatic specimen removed 31 days after the first operation (after about one week only of glycosuria) showed the islands unchanged except for vacuolation in a few of the cells. Meantime the remnant had hypertrophied considerably, so that even after removal of 0.5 gm. of additional tissue the diabetes only gradually became severe. Thirty-eight days after the second operation the islands were found extremely vacuolated and evidently somewhat reduced in size. For a period of fifty-five days following the third operation insulin was given in such a manner that glycosuria was sometimes absent and sometimes present to moderate degree. A specimen removed at the end of this time showed the islands apparently unchanged in number, and with a minority of their cells slightly vacuolated and the majority normal in size and granulation.

### *Explanation of Figures*

Figure 1 is a photograph of a typical island of dog No. 195, in the pancreatic tissue removed just before insulin treatment. The hydropic changes have reached a degree which would mean a

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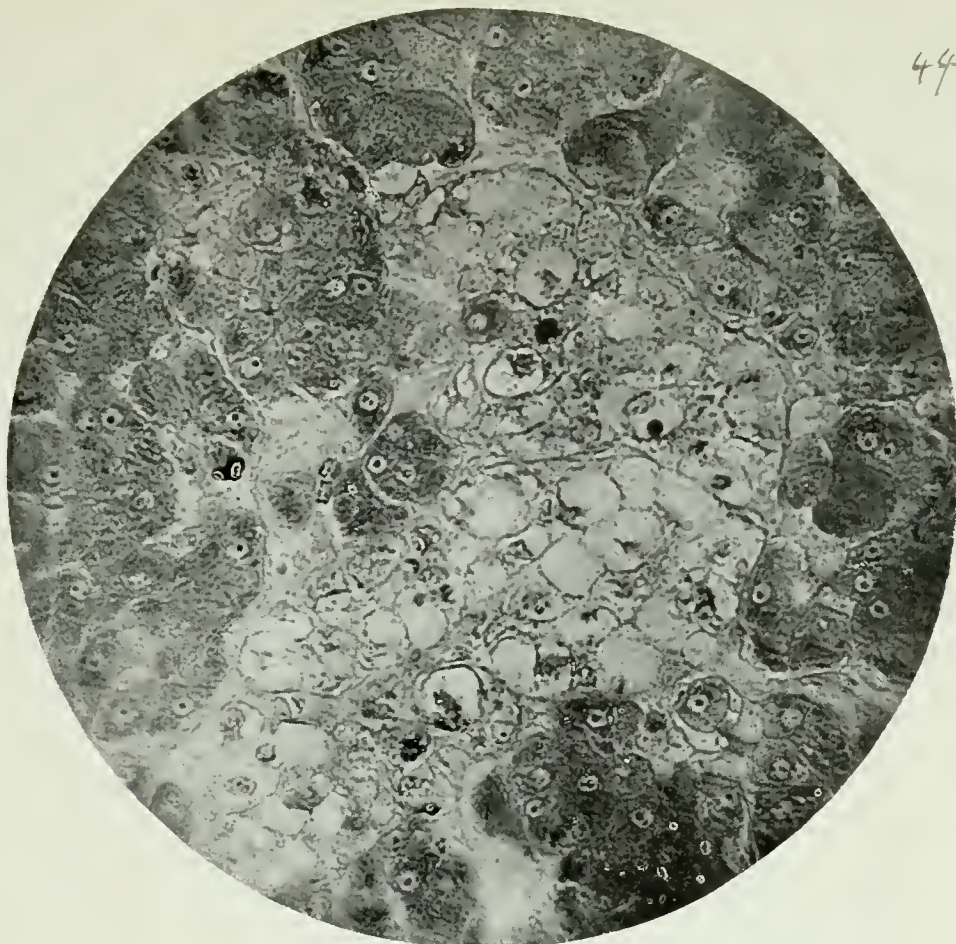


Fig. 1

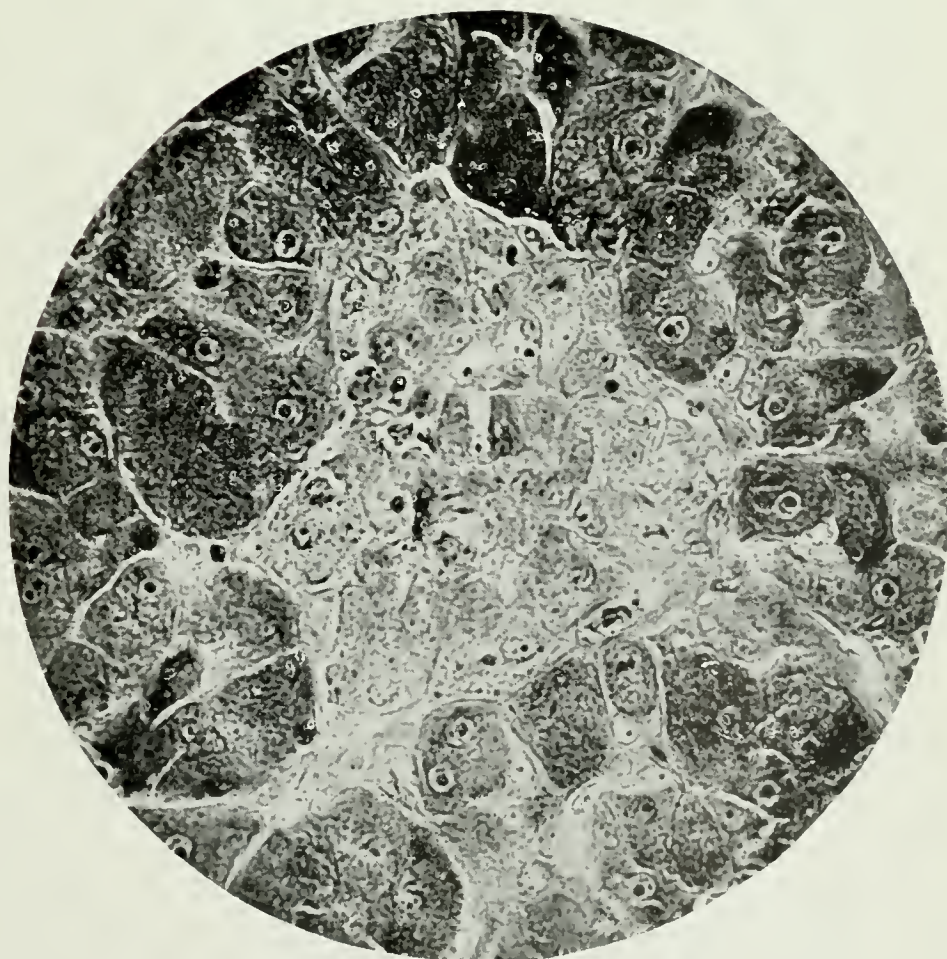


Fig. 2







very great reduction of total island tissue if the vacuolated cells should be lost.

Figure 2 shows a typical island after 15 days of symptomatic control by insulin. The amount of island tissue is not visibly reduced, and the cells are free from vacuolation. The only clear spaces in the island are capillaries.

### *Discussion*

1. *Unsuccessful experiments.*—About twenty-five dogs were used for this investigation. As the rate of hydropic degeneration varies with the intensity of the diabetes, and as this intensity cannot be exactly gauged, some experiments were lost by the wrong timing of operations, either too soon or too late for the degree of degeneration that was desired. The general time limits outlined by Allen held good. As usual, a few diabetic animals died from distemper and other accidental causes. The greatest difficulty consisted in the regulation of the insulin dosage. Not only daily urinalyses and frequent blood sugar tests, but also close watching of the animals themselves by day and night, were required in order to keep them in safe condition without glycosuria or any marked hyperglycemia. On account of changes of tolerance, doses of insulin which formerly had proved necessary caused fatal hypoglycemia in several instances, in spite of this watchfulness. Sudden acidosis was also a troublesome cause of fatalities. Details will be given in a later publication.

2. *Diet.*—The most convenient diet for breaking down tolerance and bringing on severe diabetes was naturally one high in carbohydrate. Under insulin treatment a weighed diet was necessary for accurate control, and large excursions of the blood sugar were best avoided by feeding three daily meals of lean meat. In a number of instances, however (such as dogs No. 148 and 164), the mixed bread and meat diet was continued, thus excluding any criticism that the process in the islands might be altered by mere change of diet rather than by control of diabetic symptoms.

3. *Condition of pancreatic parenchyma.*—Apart from the small zone of fibrosis adjoining the operative scar in the pancreas, the acinar tissue was always found normal in all respects. In particular, it showed no visible changes in connection with the presence or absence of diabetic symptoms. No appearances of "transi-

tions" between acinar and island tissue were found. No granule stains were made to distinguish alpha and beta cells, as we rested upon the existing information that only the beta cells in the islands become vacuolated in diabetes. Vacuolation observed occasionally in centro-acinar cells and in the small ducts or "cell cords" seems to bring these cells into some relation with the beta cells.

4. *Other interpretations of results.*—It might be assumed that the vacuolated cells go to pieces and are replaced by new-formed cells or islands. But we have a number of observations, as in dog No. 226, indicating that destroyed cells or islands are not replaced on any large scale. The capacity for hyperplasia and corresponding increase of food tolerance belongs to the early stage of diabetes, and in the late severe stage not only the functional power but also the regenerative power of the insular tissue seems to be exhausted. In particular, any such extensive regeneration in such a short time as two weeks can be safely excluded.

5. *Insulin requirements of dogs.*—The absolute requirement shown, namely from 25 to 74 units per day for dogs weighing from 21 to 36 pounds, is surprising in comparison with the requirements both of human patients and of the totally depancreatized dog studied by Bliss.<sup>2</sup> The dog has relatively a higher metabolism than man, also a higher body temperature, and the glucose tolerance bears comparison with that of man in absolute quantities rather than in relation to weight. The seeming paradox that a partially depancreatized dog requires more insulin than a totally depancreatized dog is explained by the ability to digest food and the consequent higher nutrition. There are already some reasons to suppose that the completely vacuolated island cells are functionally exhausted and useless. This view will be strengthened if a recovery of tolerance, i.e., a reduced need for administered insulin, accompanies the recovery of granulation by the beta cells. This is the actual occurrence in the experiments. The absolute insulin production by the remnant cannot be accurately estimated, because of changing conditions of nutrition and unknown variables. For the same reason the insulin requirement of different dogs differs. Just as in human patients,<sup>3</sup> it is influenced in every individual by the severity of diabetes, diet, body weight, etc., but it cannot be predicted for different

individuals according to any fixed law. But the general reduction of insulin requirement as the island cells recover granulation seems significant.

### *Conclusions*

1. The hydropic degeneration of the beta cells of the islands of Langerhans is reversible when the functional strain is relieved. The vacuolated cells recover normal form and granulation.

2. As the development of the degeneration is slow, the recovery is also slow, requiring approximately two weeks for its completion. An incidental inference is that the cells must form their own secretion, and do not take up injected insulin from the blood for storage in the form of granules.

3. These observations strengthen the proof that the hydropic degeneration of islands is strictly the result of functional overstrain.

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# MINERAL METABOLISM IN RATS UNDER THE INFLUENCE OF B AND D VITAMINES

By CASIMIR FUNK and AUGUSTUS LEVY\*

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Very few metabolism experiments have been made on animals for the purpose of studying the influence of vitamine B on the mineral metabolism. Schaumann<sup>1</sup> reported the results of a metabolism experiment on four pigeons, in which he studied the increase in food intake and the influence on the balance of nitrogen, phosphorus, calcium and magnesium, after the administration of B vitamine. The basal diet consisted of polished rice and as the source of B vitamine an extract of rice-polishings was used. The experiment, which lasted 20 days, can be summarized in the following table:

	Nitrogen	P <sub>2</sub> O <sub>5</sub>	CaO	MgO
Vitamine-free diet .....	—0.942	—0.433	—0.138	+0.0025
Plus Vitamine B.....	+0.0042	—0.294	+0.0018	+0.0042

There are, however, no experiments reported in the literature which deal with the action of carefully purified B vitamine, and there are no experiments dealing with the action of the yeast—growth-promoting, or D vitamine, on the metabolism of rats.

In the present investigation, three normal male adult rats of the same litter were used. They were placed in metabolism cages, devised by Macallum,<sup>2</sup> which prevent the scattering of the food and permit the quantitative separation of feces and urine.

The basal food, as well as all the vitamine additions, were analyzed for nitrogen, phosphorus, sulphur and calcium. The metabolism experiment covered a period of 27 days and included a period of diet free from vitamine B and D (but containing vitamine A in form of butter, a period in

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\* The work was performed at the Biochemical Laboratory of the College of Physicians and Surgeons, and the Research Laboratory of H. A. Metz, New York City. During the work we enjoyed the co-operation of Dr. Julia B. Paton, which we wish to acknowledge here.

which vitamine D was given, followed by vitamine B and finally the mixture of both. The experiment was finally concluded by a short period identical with the first period; this means on a diet devoid of vitamine B and D.

The details of the experiment can be followed from the appended tables and the chart summarizing the results.

The food used consisted of the following materials:

18 gm. casein,	
18 " butter,	
6 " lard,	
54 " starch,	
4 " salt mixture (according to Osborne and Mendel).	
100 gm. of food contained:	

	Calculated	Analyzed
Nitrogen .....	2.83	2.72
Phosphorus .....	0.35	0.40
Sulphur .....	0.17	0.27
Calcium .....	0.33	0.64

Here the differences noted between the amount calculated and actually obtained on analysis emphasize the importance of not relying on tables for calculating the chemical composition of foods in metabolism experiments.

The vitamine additions were also analyzed for the chemical elements under investigation. They contained mere traces of sulphur and calcium, traces which could not be adequately determined. For nitrogen and phosphorus, the following figures were obtained:

	Nitrogen	Phosphorus
3.0 cc. of vitamine B.....	.0021	0.0000
0.5 cc. of vitamine D.....	.0028	.00005

Vitamine B was a highly purified preparation from yeast, previously tested on pigeons and containing only traces of vitamine D. The D vitamine was also obtained from yeast and freed from B by fractional absorption by means of fuller's earth. It was tested both on pigeons (for absence of vitamine B) and for its potency to allow the yeast cells to grow. The vitamine solutions were given in separate containers.

As regard analytical methods, the nitrogen was determined by Kjeldahl, the phosphorus by the Pemberton-Neumann method and the sulphur and calcium by using a fusion mixture and then proceeding in the usual manner. As regard urine, which amounted daily to about 15 cc., the funnel and container were washed up to 100 cc. and aliquot parts were taken for the various determinations. The feces were collected for the whole period, dried and analyzed. The nitrogen and phosphorus of urine and feces were determined by Kjeldahl and Pemberton-Neumann methods, respectively. The sulphur in the urine was analyzed by the method devised by Folin for determination of total sulphur and in the feces after fusion. The calcium in the urine was determined according to McCrudden, and in the feces after fusion as oxalate and weighed as calcium oxide. All analyses were made in duplicates.



TABLE I

Rat No. 7253, Male. Born July 2, 1921

Date	Body Wgt.	Food In-take gm.	Vitamine	N In-take gm.	P In-take gm.	S In-take gm.	Ca In-take gm.	Urine N gm.	Urine P gm.	Urine S gm.	Urine Ca gm.	Feces in dry basis	Feces N gm.	Feces P gm.	Feces S gm.	Feces Ca gm.	N Balance gm.	P Balance gm.	S Balance gm.	Ca Balance gm.
Mar. '22																				
1				.299	.044	.030	.070	.125	.0144	.031	.017						+.134	+.011	—	+.023
2	405			.299	.044	.030	.070	.256	.0205	.021	.011						+.003	+.004	—	+.029
3	372			.299	.044	.030	.070	.228	.0207	.019	.004						+.031	+.004	.000	+.036
4				.299	.044	.030	.070	.310	.0214	.025	.019					.210	—	+.003	—	+.021
5				.299	.044	.030	.070	.281	.0211	.027	.025						—	+.004	—	+.015
6				.408	.060	.041	.096	.298	.0194	.026	.008						+.070	+.021	+.004	+.058
7				.462	.068	.046	.109	.244	.0181	.021	.004						+.178	+.031	+.014	+.075
8	373			.381	.056	.038	.090	.244	.0232	.023	.015						+.119	+.017	+.010	+.060
9			2 cc of D	.435	.064	.044	.102	.308	.0225	.020	.010						+.109	+.025	+.019	+.077
10			"	.381	.056	.038	.090	.272	.0228	.022	.016					.090	+.091	+.017	+.011	+.059
11	376		"	.490	.072	.049	.115	.243	.0169	.012	.010						+.229	+.039	+.032	+.090
12			"	.109	.016	.011	.026	.178	.0220	.029	.012						—	—	—	—
13			Omitted	.462	.068	.046	.109	.343	.0240	.037	.022						+.101	+.028	+.004	+.072
14			3 cc of B	.653	.096	.066	.154	.293	.0218	.026	.026						+.334	+.052	+.035	+.118
15	384		"	.381	.056	.038	.090	.283	.0256	.026	.024						+.072	+.009	+.008	+.056
16			"	.435	.064	.044	.102	.238	.0229	.021	.006					.052	+.171	+.019	+.018	+.086
17			"	.707	.104	.071	.166	.336	.0286	.029	.022						+.345	+.053	+.038	+.134
18	378		"	.490	.072	.049	.115	.265	.0224	.018	.027						+.199	+.028	+.027	+.078
19			3 cc B; 0.5 cc D	.626	.092	.063	.147	.351	.0258	.024	.004						+.257	+.053	+.036	+.137
20			"	.408	.060	.041	.096	.301	.0206	.025	.031						+.089	+.027	+.013	+.059
21			"	.789	.116	.079	.186	.295	.0258	.022	.010					.034	+.476	+.077	+.052	+.170
22	379		"	.299	.044	.030	.070	.214	.0200	.014	.009						+.067	+.011	+.013	+.055
23			"	.408	.060	.041	.096	.316	.0204	.019	.021						+.074	+.026	+.019	+.069
24			"	.381	.056	.038	.090	.325	.0249	.022	.013						+.038	+.018	+.014	+.071
25	379		No Vitamines	.462	.068	.046	.109	.292	.0234	.020	.008					.054	+.148	+.030	+.017	+.083
26			"	.598	.088	.060	.141	.320	.0234	.022	.012						+.251	+.050	+.029	+.111
27	375		"	.163	.024	.016	.038	.315	.0216	.021	.010						—	—	—	+.010

\* Food cup not filled by mistake.

TABLE II

Rat No. 7255, Male. Born July 2, 1921

Date	Body Wgt. gm.	Food In-take gm.	Vitamine	N In-take gm.	P In-take gm.	S In-take gm.	Ca In-take gm.	Urine N gm.	Urine P gm.	Urine S gm.	Urine Ca gm.	Feces in gm., dry basis	Feces N gm.	Feces P gm.	Feces S gm.	Feces Ca gm.	N Balance gm.	P Balance gm.	S Balance gm.	Ca Balance gm.
Mar., '22																				
1	412			.218	.032	.022	.051	.153	.0204	.018	.022						+.039	-.003	.000	+.015
2				.218	.032	.022	.051	.202	.0212	.016	.012						-.010	-.004	+.002	+.024
3				.218	.032	.022	.051	.157	.0184	.013	.001						+.045	-.001	+.004	+.037
4	374	32		.218	.032	.022	.051	.363	.0293	.025	.035	4.90	.180	.1004	.033	.096	-.171	-.012	-.008	+.002
5		15		.408	.060	.041	.096	.298	.0226	.024	.021						+.084	+.023	+.011	+.061
6		11		.299	.044	.030	.070	.196	.0126	.018	.018						+.077	+.017	+.008	+.038
7		9		.245	.036	.025	.058	.344	.0223	.029	.008						-.125	-.001	-.008	+.036
8	373	14	2 cc of D	.381	.056	.038	.090	.206	.0218	.013	.022						+.150	+.015	+.020	+.057
9		17	"	.462	.068	.046	.109	.241	.0187	.023	.010	4.6	.150	.1199	.037	.066	+.196	+.030	+.017	+.088
10		11	"	.299	.044	.030	.102	.319	.0263	.014	.017						-.045	-.002	+.010	+.042
11	368	16	"	.435	.064	.044	.102	.220	.0115	.017	.021						+.190	+.033	+.020	+.069
12		7*	"	.190	.028	.019	.045	.416	.0220	.043	.024						-.251	-.014	-.030	+.010
13		19	Omitted	.517	.076	.052	.123	.392	.0303	.037	.017						+.100	+.026	+.009	+.095
14		24	3 cc of B	.653	.096	.066	.154	.361	.0279	.036	.031						+.266	+.052	+.026	+.117
15	378	17	"	.462	.068	.046	.109	.375	.0329	.033	.022						+.061	+.019	+.009	+.081
16		16	"	.435	.064	.044	.102	.283	.0218	.028	.017	4.3	.130	.0788	.020	.032	+.126	+.027	+.012	+.079
17		21	"	.571	.084	.057	.134	.361	.0303	.030	.018						+.184	+.038	+.023	+.110
18	382	18	"	.490	.072	.049	.115	.404	.0326	.026	.017						+.060	+.024	+.019	+.092
19		22	3 cc B; 0.5 cc D	.598	.088	.060	.141	.235	.0207	.019	.003						+.345	+.055	+.038	+.129
20		11	"	.299	.044	.030	.070	.249	.0165	.016	.029						+.032	+.015	+.011	+.032
21		11	"	.299	.044	.030	.070	.273	.0237	.019	.012						+.008	+.009	+.008	+.049
22	370	10	"	.272	.040	.027	.064	.309	.0216	.023	.018	2.95	.110	.0744	.017	.053	-.055	+.006	+.001	+.037
23		19	"	.517	.076	.052	.123	.287	.0244	.027	.029						+.212	+.039	+.022	+.085
24		19	"	.517	.076	.052	.123	.396	.0271	.030	.018						+.103	+.037	+.019	+.096
25	380	20	No Vitamines	.544	.080	.054	.128	.368	.0249	.030	.024						+.143	+.023	+.013	+.088
26		28	Except A	.762	.112	.076	.180	.337	.0231	.028	.016	2.90	.100	.0973	.032	.046	+.392	+.057	+.037	+.148
27	389	12	"	.326	.048	.033	.077	.331	.0224	.027	.013						-.038	-.007	-.005	+.049

\*Food cup not filled by mistake.

TABLE III

Rat No. 7260, Male. Born July 2, 1921

Date	Body Wgt. gm.	Food In-take gm.	Vitamine	N In-take gm.	P In-take gm.	S In-take gm.	Ca In-take gm.	Urine N gm.	Urine P gm.	Urine S gm.	Urine Ca gm.	Feces in gm., dry gm.	Feces N gm.	Feces P gm.	Feces S gm.	Feces Ca gm.	N Balance gm.	P Balance gm.	S Balance gm.	Ca Balance gm.
Mar., '22																				
1	375			.245	.036	.025	.058	.164	.0141	.021	.015						+.022	+.006	-.002	+.033
2				.245	.036	.025	.058	.266	.0210	.022	.010						-.080	-.001	-.003	+.038
3				.245	.036	.025	.058	.192	.0192	.022	.003						-.006	+.001	-.003	+.045
4	353	37		.272	.040	.027	.064	.322	.0221	.024	.030		.410	.1129	.0433	.069	-.109	+.002	-.003	+.024
5		8		.218	.032	.022	.051	.307	.0196	.026	.027						-.148	-.004	-.010	+.014
6		28		.763	.112	.077	.180	.325	.0194	.024	.011						+.378	+.077	+.043	+.159
7		14		.381	.056	.038	.090	.246	.0183	.024	.009						+.076	+.022	+.008	+.070
8	365	13	2 cc of D	.354	.052	.036	.083	.209	.0216	.018	.018						+.123	+.008	+.011	+.052
9		15	"	.408	.060	.041	.096	.281	.0205	.021	.001						+.105	+.017	+.014	+.082
10		15	"	.408	.060	.041	.096	.295	.0201	.021	.017		.130	.1323	.039	.081	+.091	+.018	+.014	+.066
11	360	18	"	.490	.072	.049	.115	.236	.0213	.017	.014						+.232	+.029	+.026	+.088
12		4*	"	.109	.016	.011	.026	.288	.0166	.046	.043						-.201	-.023	-.041	-.031
13		13	Omitted	.354	.052	.036	.083	.305	.0200	.027	.008						+.028	+.010	+.003	+.062
14		21	3 cc of B	.571	.084	.057	.134	.152	.0176	.025	.024						+.397	+.045	+.024	+.098
15		17	"	.462	.068	.046	.109	.274	.0238	.025	.020						+.166	+.023	+.014	+.077
16	366	14	"	.381	.056	.038	.090	.269	.0258	.025	.025		.110	.1048	.034	.058	+.090	+.009	+.006	+.053
17		15	"	.408	.060	.041	.096	.297	.0233	.028	.027						+.089	+.016	+.007	+.057
18	366	16	"	.435	.064	.044	.102	.280	.0209	.022	.015						+.133	+.022	+.015	+.075
19		21	3 cc B; 0.5 cc D	.571	.084	.057	.134	.217	.0217	.021	.006						+.331	+.046	+.032	+.120
20		16	"	.435	.064	.044	.102	.370	.0167	.025	.039						+.042	+.031	+.014	+.055
21		14	"	.381	.056	.038	.090	.280	.0198	.020	.009						+.078	+.019	+.014	+.073
22	368	14	"	.381	.056	.038	.090	.342	.0244	.023	.011						+.016	+.015	+.011	+.071
23		19	"	.517	.076	.052	.123	.364	.0309	.025	.023						+.130	+.028	+.023	+.092
24		17	"	.462	.068	.046	.109	.347	.0214	.031	.024						+.092	+.030	+.011	+.077
25	375	20	No Vitamines	.544	.080	.054	.128	.356	.0243	.024	.013						+.155	+.030	+.023	+.102
26		23	Except A	.626	.092	.063	.147	.313	.0194	.025	.011						+.280	+.047	+.031	+.129
27	374	8	"	.218	.032	.022	.051	.307	.0183	.024	.010						-.122	-.012	-.009	+.034

\* Food cup not filled by mistake.



*Discussion*

In comparing the charts which summarize the results obtained, the following deductions appear justified. The experimental period, which included only the vitamine A addition to the diet, rendered the nitrogen balance negative very quickly. It had a little less influence on the phosphorus balance, and still less on the sulphur, while the calcium balance never became negative. The phosphorus balance then was the one which followed nitrogen most closely. The administration of D vitamine improved the mineral balance greatly, but not so much when vitamine B was given in addition to A vitamine. It is, however, possible that we had to deal with a storage of vitamine D from the previous period and therefore a combined action of B and D. This conception is born out to some extent by the next period (especially as regard P, S and Ca), in which B and D were given together.

When we come now to the food intake, we find that vitamine D had less effect on the appetite as compared with its action on the mineral balance. On the other hand, vitamine B had considerable effect in both directions. This result corroborates the control of the purity of both preparations used, namely that vitamine D was free from B and B almost free from D. With vitamine D there was very little action on the appetite and distinct influence on the mineral metabolism, while with vitamine B we had both actions manifested. This is particularly important for the consideration of the fourth period, where B and D were given together, and where D was given in  $\frac{1}{4}$  the amount used during the second period when D was given alone.

The results obtained point strongly to the possibility of vitamine D playing a part in the metabolism of the rat, in accordance with the conception of Funk and Dubin.<sup>3</sup> Recently Levene and Muhlfeld<sup>4</sup> came to the conclusion that the antiberiberi vitamins for rats and pigeons do not appear to be identical. These and previous results of ours speak rather in favor of the conception that rats and pigeons require one and the same vitamine B, but rats, unlike the pigeons, require at least one other vitamine. Our results emphasize particularly the importance of vitamins B and D for the mineral metabolism.

### *Conclusions*

The addition of vitamins B and D to an artificial diet, containing vitamin A, serves to improve the mineral balance very greatly. Although vitamin D has little influence on the food intake, it improves the mineral balance markedly, while vitamin B exhibits both actions. The results emphasize once more the possible importance of vitamin D for the metabolism of the rat.

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## STUDIES ON THE CHEMISTRY OF COD LIVER OIL\*

### I. THE EFFECT OF HYDROGENATION UPON THE VITAMINE CONTENT.

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Fish oils have been used in medicine for many years. Various investigators have reported on the value of cod liver oil in rheumatism, gout, osteomalacia and rickets, but it appears that little credence was placed in their findings. In view of the proven beneficial effect of cod liver oil in rickets, it is remarkable that it was held so lightly by the medical profession. The laity, on the contrary, clung tenaciously to a product, the use of which gave such tangible results.

The development of the vitamine theory and the discovery of the fat-soluble vitamins provided a marked impetus to research in nutrition and a voluminous literature has been built up on this subject. Without referring to any particular publication, it will suffice to say that within the last few years it has been definitely established that cod liver oil is a specific for rickets and for ophthalmia, both as a cure and as a preventive. Further, the therapeutic value of the oil is due to the vitamins contained therein, and not to the oil, *per se*.

Our attention, therefore, turned to the preparation of a concentrate which would contain the bulk of the vitamins, and which could be used as a starting point for further chemical fractionation. Because of the relative instability of the vitamins of cod liver oil, especially when exposed to oxidation, it occurred to us to subject the oil to hydrogenation.

A perusal of the literature reveals comparatively little dealing with the hydrogenation of cod liver oil. Drummond,<sup>1</sup> working

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\* This investigation was started in January 1922 and is still being continued. A preliminary communication was presented before the Society for Experimental Biology and Medicine, Dec. 19, 1923, at Columbia University.

with whale oil hydrogenated at 250°C. for 4 - 6 hours, found that the hardened oil was entirely deficient in "fat-soluble A," which at that time was thought to be synonymous with "antirachitic vitamine." Since then, however, the weight of evidence has tended to prove that cod liver oil contains two vitamines, antirachitic and antiophthalmic. The latter is now designated as "fat-soluble A," or "Vitamine A," the antiophthalmic growth-promoting vitamine.\*

Inasmuch as Drummond's animal experiments were so arranged that growth was taken as an index of the vitamine activity of the hydrogenated whale oil, the results point merely to the destruction of the fat-soluble, growth-promoting vitamine A; they have no significance as regards the antirachitic vitamine. That the antiophthalmic vitamine A was destroyed is not to be wondered at when one considers its instability and the high temperature at which the oil was hydrogenated.

Paal and Roth,<sup>2</sup> in 1908, when vitamines were still unknown, hydrogenated butter and cod liver oil while engaged in a study of the catalytic action of colloidal metals of the platinum group. The process used by these investigators is a very mild one. As modified to suit our needs, it is carried out at about 55°C. for approximately 36 hours. This is in sharp contrast to the drastic hydrogenation reported by Drummond.

Employing both the curative and preventive type of experiment, the various fractions obtained by us, and about to be described, were tested on more than 150 rats. The diets used included a synthetic diet practically free from antirachitic vitamine, the McCollum diet No. 3143, the Pappenheimer-Zucker diet D, and a synthetic diet (Diet A) free from antirachitic and antiophthalmic vitamine.

The detailed experimental procedure has been outlined in the second paper<sup>3</sup> of this series. In order to avoid an unusually lengthy publication we shall confine ourselves to just such representative data as will be descriptive of our results.

Fraction I. — This fraction, which possesses no vitamine activity, is the aqueous reduction mother-liquor after it has been

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\* One is frequently at a loss to decide just what an author has in mind when he says, "cod liver oil was used as a source of fat-soluble A." All possibility of confusion would be avoided if the specific terms, antiophthalmic and antirachitic, were used to classify the fat-soluble vitamines.

concentrated down to an amount corresponding to the original quantity of oil hydrogenated.

Fraction II. — The hydrogenated cod liver oil, obtained as a snow-white product, is odorless and tasteless, and melts at about 55°C. The yield is about 95 gm. from 100 gm. cod liver oil. When used in amounts corresponding to 0.100 gm. cod liver oil daily, it prevents and cures rickets. Smaller dosage was not tried.

Fraction III. — This substance is the residue left after extracting fraction II with alcohol. The yield is about 90 gm. from 95 gm. of fraction II. Fraction III is inactive in amounts corresponding to 0.250 gm. cod liver oil daily. Larger doses were not tried.

Fraction IV. — This is a creamy-white product almost odorless and tasteless, melting at about 30°C., extracted from fraction II by means of alcohol. The yield is about 4.5 gm. from 95 gm. fraction II. When given in amounts equivalent to 0.100 gm. cod liver oil daily, it prevents and cures rickets. Smaller dosage was not tried. Fraction IV may also be extracted from fraction II by means of acetic acid.

Fraction IVa. — This is the same as fraction IV, resembling it in appearance, but with the cholesterol removed. Yield is about 0.045 gm. from 4.5 gm. fraction IV. Administered in amounts corresponding to 0.250 gm. cod liver oil daily, it prevents and cures rickets. Smaller dosage was not tried.

Fraction C. — The cholesterol fraction proved to be inactive.

In the few cases in which the above active fractions were tested in attempts to cure ophthalmia complete cures were not obtained, though improvement was noted. However, we do not conclude that hydrogenation destroys the antiophthalmic vitamin completely, inasmuch as growth is a better index than ophthalmia, and our animals grew fairly well.

In this connection, it is of interest to note that Goldblatt and Zilva,<sup>4</sup> using the preventive type of experiment with animals fed on the McCollum diet No. 3143, found that 33.7 mg. daily of a hardened cod liver oil protected against rickets, and that 1.8 mg. promoted growth. The original oil, before hardening, also promoted growth in daily doses of 1.8 mg. The antirachitic value of the original oil was not determined.

In a later publication, Zilva<sup>5</sup> reports on the stability of vitamin A of cod liver oil when the latter is subjected to hydro-



genation at a temperature of 150° - 175°C. for a period varying from 50 minutes to 3 hours and 5 minutes.

In the light of these results, it is of interest to record the observation that in our hydrogenation process, the oil hardens almost completely in a few hours, the reduction vessel being evacuated before the introduction of hydrogen. The low temperature together with the short period of hydrogenation would undoubtedly yield a very potent product.

From the above, it appears that the vitamine potency of cod liver oil is not appreciably destroyed by a suitable hydrogenation process.

It is thus possible to prepare a potent concentrate which may be used as a starting point for further chemical fractionation, and as a source of fat-soluble vitamins possessing obvious advantages over fresh cod liver oil.

Further work along this line, described in the second communication<sup>3</sup> of this series, has resulted in the elaboration of a superior method of preparing a concentrate which is active in concentrations of 1 in 10,000.

The antirachitic value of hydrogenated cod liver oil is illustrated by the accompanying sections and radiographs.\*

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#### EXPLANATION OF FIGURES

All fractions administered daily from the beginning of the experiment (except where otherwise indicated) in amounts corresponding to 0.100 gm. fresh cod liver oil. Radiographs made at the end of the experiment.

Fig. 1. Rat 95. McCollum Diet 3143 for 42 days. Fraction IV - a, (alcoholic extract of hydrogenated cod liver oil) given during the last 10 days. Section of tibia shows deposition of calcium.

Fig. 2. Rat 107. Control. Same dietary conditions as above. Section shows typical marked rickets.

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\* We are indebted to Reuben H. Gross for all the X-ray work.

Fig. 3. Rat 458. Control. Diet D for 50 days. Rib shows marked rickets.

Fig. 4. Rat 458. Radiograph shows marked rickets.

Fig. 5. Rat 388. Diet D for 30 days. Fraction C-6 (fresh cod liver oil). Rib shows no rickets.

Fig. 6. Rat 388. Radiograph showing no rickets.

Fig. 7. Rat 437. Diet D for 41 days followed by Diet S. R. 4 for 13 days. Fraction C-23 (hydrogenated cod liver oil, similar to Fraction II). Rib shows no rickets.

Fig. 8. Rat 437. Radiograph shows no rickets.

Fig. 9. Rat 446. Dietary conditions same as in Rat 437. Fraction C-23. Rib shows no rickets.

Fig. 10. Rat 446. Radiograph shows no rickets.

Fig. 11. Rat 513. Diet S. R. 4 for 48 days. Fraction C-23 started on the 14th day. Radiograph shows no rickets.

Fig. 12. Rat 513. Radiograph shows no rickets.

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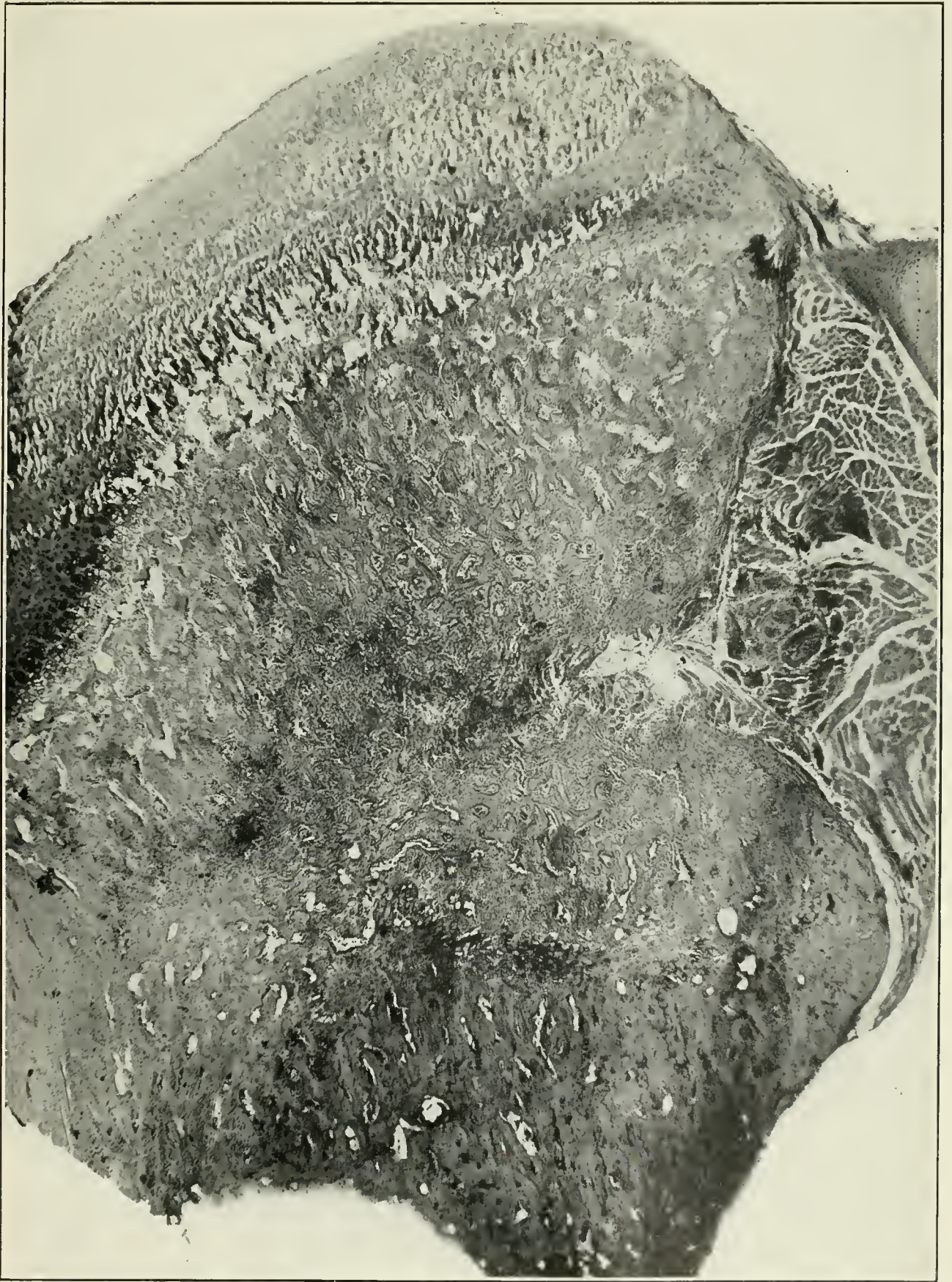


FIG. 1







FIG. 2





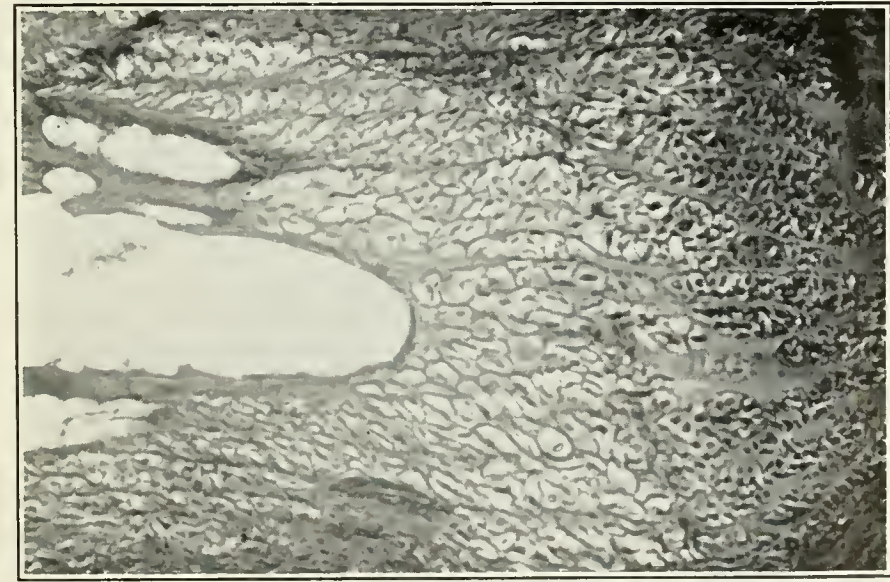


FIG. 3

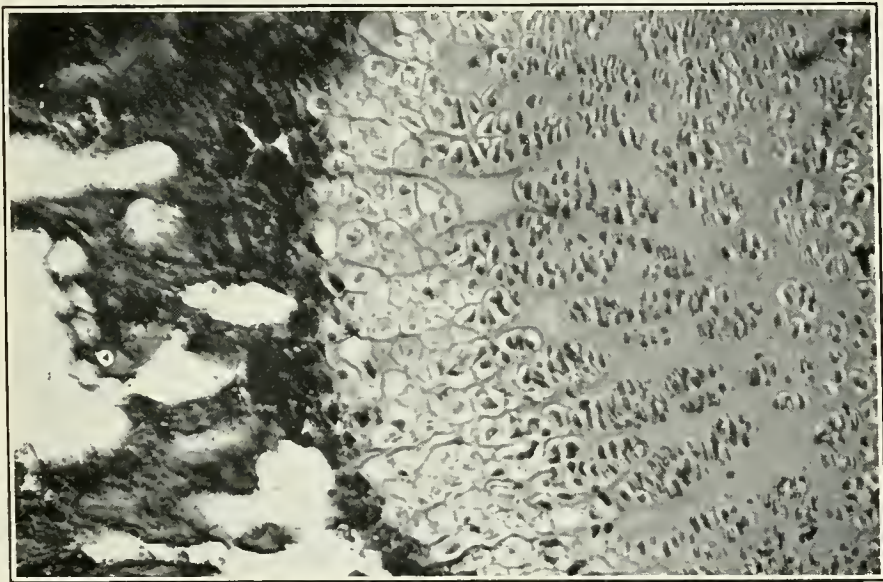


FIG. 5

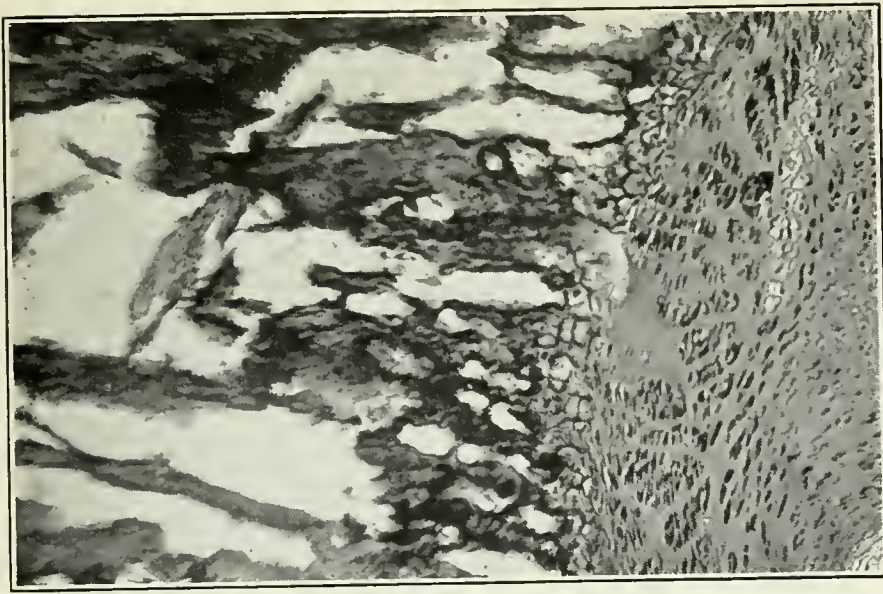


FIG. 7

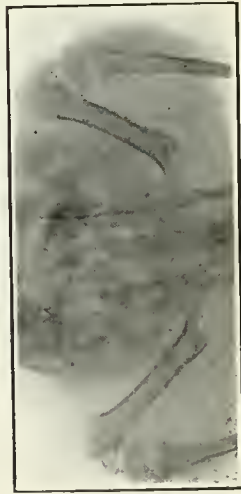


FIG. 4

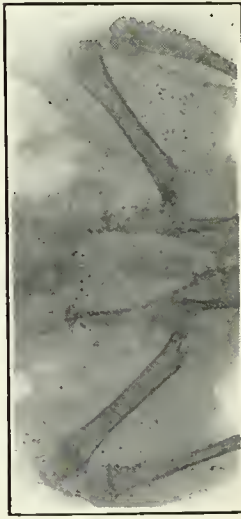


FIG. 6

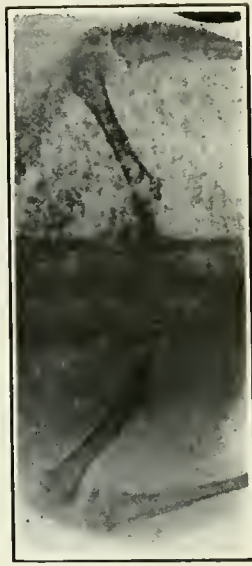


FIG. 8





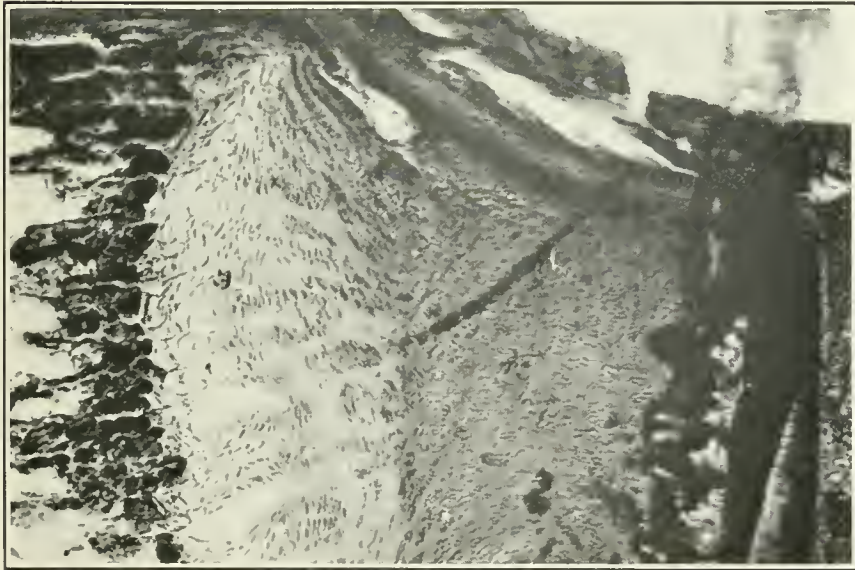


FIG. 9

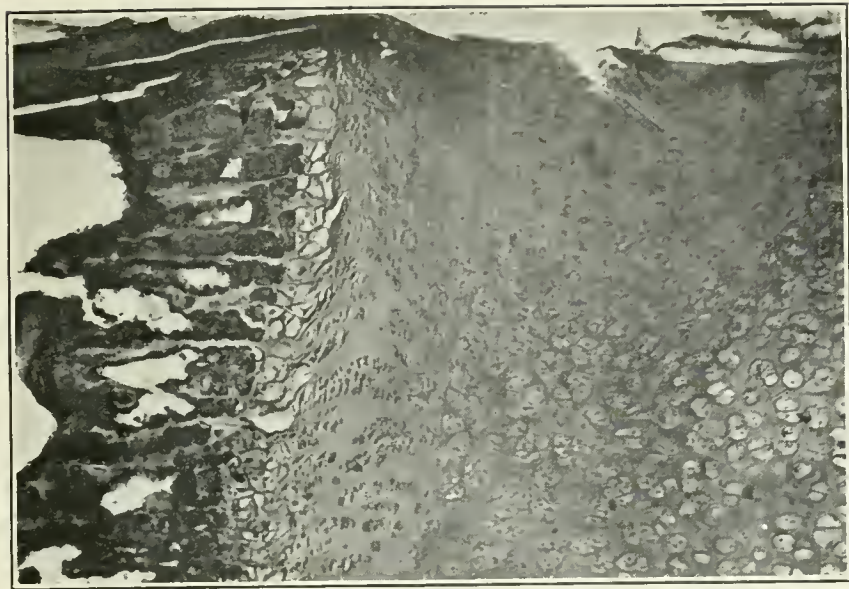


FIG. 11



FIG. 10

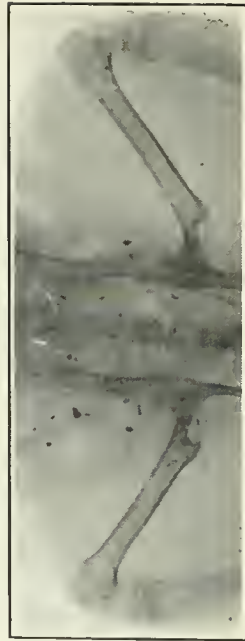


FIG. 12



## STUDIES ON THE CHEMISTRY OF COD LIVER OIL\*

### II. A COD LIVER OIL CONCENTRATE MANIFESTING BOTH ANTI-RACHITIC AND ANTIOPHTHALMIC PROPERTIES.

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In recent years, the demonstration of the specificity of cod liver oil for rickets has resulted in renewed efforts to isolate and identify the active principle of the oil. Though the goal has not been reached, considerable progress has been reported by many investigators.

The problem has been attacked in various ways. Simple extraction with a variety of solvents has yielded rather indifferent results. By means of hydrogenation followed by a suitable extraction and purification we<sup>1</sup> have prepared an active substance 1000 times more concentrated than the original cod liver oil. However, the most promising finding has been the demonstration that the vitamins known to be present in cod liver oil — the antirachitic and antiophthalmic — are to be found in the ether-soluble, non-saponifiable portion of the oil.

On saponifying cod liver oil directly with alcoholic KOH, Steenbock, Sell and Buell,<sup>2</sup> Steenbock, Nelson and Hart,<sup>3</sup> and Steenbock, Jones and Hart<sup>4</sup> obtained a product that was curative for both rickets and ophthalmia. Using an analogous process, similar results were reported by Zucker, Pappenheimer and Barnett<sup>5</sup> and also by Takahashi.<sup>6</sup> Some time later, Zucker<sup>7</sup> extracted cod liver oil with alcohol and then saponified the alcoholic extract. He thus obtained a product which contained only the antirachitic vitamin, the antiophthalmic vitamin having been destroyed in the procedure.

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\* This investigation was started in January 1922 and is still being continued. A preliminary communication was presented before the Society for Experimental Biology and Medicine, May 24, 1924, at Yale University.



In order to avoid the necessity for manipulating large quantities of oil, our own efforts were directed toward finding a solvent which would extract the antirachitic and antiophthalmic vitamins and leave the bulk of the oil behind. After numerous trials we found that extraction of cod liver oil with an organic acid, such as acetic or formic, served the purpose as nothing else would.

Thus, extraction of 1000 gm. cod liver oil with acetic acid yields about 50 gm. oil containing practically all of the vitamins present in the original oil. The residual oil is almost entirely free from vitamins.

Upon saponification of the above 50 gm. acid extract, we obtain 0.5 gm. concentrate — a brown syrupy mass which, on standing, crystallizes in light yellowish-brown, needle-like crystals radiating from a central point. This highly concentrated substance, which is insoluble in water but freely soluble in the ordinary organic solvents, contains practically all of the antirachitic and antiophthalmic vitamins originally present in the fresh cod liver oil.

On eliminating cholesterol and other inert substances, the above 0.5 gm. concentrate is reduced to 0.1 gm. — a concentration of 1 in 10,000. By working with extreme care, it is possible to obtain an active product showing a concentration of 1 in 15,000. During the entire procedure, suitable precautions are taken against the destructive action of oxidation.

That the concentrate may be hydrogenated without appreciable loss of activity is evident from Figs. 23 and 24. The hydrogenation was carried out by the method already described by us.<sup>1</sup>

We have additional evidence that the concentrate may be distilled with superheated steam. A highly active fraction is also obtained when the concentrate is distilled at a temperature of about 250°C. in a vacuum of about 2 mm. Experimental data along this line will appear at an early date.

Thus far, we have been unable to establish the exact chemical composition of this highly concentrated vitamin substance, since it is undoubtedly still far removed from the actual chemical entity. However, the presence of C, H and O have been demonstrated. N, S and P are absent, as are also the halogens. The  $\text{H}_2\text{SO}_4$  reaction is obtained just as with fresh cod liver oil. Further, the concentrate is not toxic even in very large doses.

*The advantages of such a concentrate are two-fold. First, it provides a convenient starting material for chemical work directed to the actual isolation and identification of the vitamins present in cod liver oil. Second, it presents an effective method of carrying out cod liver oil therapy, whether experimental or practical.*

Experiments with almost 500 rats kept upon a variety of vitamin-free and rickets-producing diets have shown that the cod liver oil concentrate described above is capable of both curing and preventing rickets and ophthalmia. It may be stated at this point that the preventive type of experiment is to be preferred to the curative.

Clinical experiments\* have revealed ample evidence that the concentrate is just as effective in children as in animals. The same conclusion was reached by Chaplin,<sup>8</sup> working with a somewhat similar concentrate. In the clinical work, the concentrate was mixed with sugar and administered in the form of 1 grain tablets, arbitrarily prepared to be the equivalent of a half teaspoonful of cod liver oil. Due to the high degree of concentration it is of course possible to prepare a small tablet containing as much or as little concentrate as may be desired for the particular experiment at hand. Necessarily, the tablets are prepared under anaerobic conditions so as to prevent loss of activity due to oxidation.

*It is our belief that infants, as early as one month after birth should be given cod liver oil if it can be tolerated; if not, the child should receive its equivalent in the form of a concentrate, as a prophylactic against rickets and other nutritive disturbances traceable to the lack of fat-soluble vitamins in the diet.*

Such a procedure would ultimately eliminate rickets as a factor in infant nutrition, and would be directly comparable with the present routine practice of giving orange juice for the prevention of scurvy.

## EXPERIMENTAL

The animals used were raised in our own laboratory.\*\* The stock diet consisted of kibbled dog biscuit and carrots, with an occasional piece of

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\* The clinical experiments were conducted by Dr. Louis Fischer, Director of the Infan-torium and Nursery of the Heckscher Foundation, New York City. His results are reported elsewhere in this issue.

\*\* We are indebted to Dr. Lafayette B. Mendel for the original breeding rats.

lettuce. From the beginning of pregnancy and throughout the period of lactation, the animals received, in addition to the stock diet, a supplementary food made up of whole milk powder 60, starch 12, lard 28. To every 100 gm. of the supplementary food, there was added 3 gm. dried brewer's yeast and 5 gm. salt mixture (Osborne-Mendel).

Usually, the animals were placed upon an experiment when they were about 4 weeks old, at which time they weighed about 40 gm. Experimental animals were kept in individual metal cages so constructed that excreta could not be eaten. Food was changed every day and the animals were weighed twice weekly.

The various cod liver oil fractions and concentrates to be tested were given apart from the diet, mixed either with some inactive oil or with sugar so as to control accurately the dosage desired. Ordinarily the fraction being tested was added to the daily portion of .2 gm. dried brewer's yeast, which was quickly consumed by the animal. Where yeast was not given separately, the substance being tested was mixed with a small quantity of food sufficient to make a thin paste. In this way, there was never any doubt but that the animal had taken the fraction completely.

The results were controlled by X-ray of the tibia\* and by microscopic examination of tibia and rib sections.

The diets used at one time or another during the course of our work are tabulated below.

Diet	S. R. 1. gm.	S. R. 2 gm.	S. R. 3 gm.	S. R. 4 gm.
Egg albumin .....	—	—	7.0	18.0
Casein .....	18.0	17.0	10.0	—
Starch .....	45.0	46.6	45.6	55.6
Lard .....	25.5	26.0	26.0	16.0
Salts (Osborne-Mendel)....	4.0	3.0	3.0	3.0
Butter .....	5.0	5.0	3.0	2.0
CaCO <sub>3</sub> .....	2.5	2.4	2.4	2.4
Dried brewer's yeast.....	0.2 (daily)	0.2 (daily)	3.0	3.0

The P and Ca content of the above diets approximate very closely the P and Ca content of the McCollum Diet No. 3143. On these diets, the animals are maintained in a relatively good state of nutrition. At the same time they develop a slightly milder form of rickets than on Diet No. 3143, which has the following composition:

*Diet 3143*

	gm.
Maize .....	33.0
Wheat .....	33.0
Wheat gluten .....	15.0

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\* We wish to express our appreciation of the courtesy of Reuben H. Gross in taking care of all the X-ray work.



Gelatin .....	15.0
NaCl .....	1.0
CaCO <sub>3</sub> .....	3.0

In studying the growth-promoting antiophthalmic activity of cod liver oil concentrate and other fractions, we found the following diet, free from all fat-soluble vitamins (Diet A), to be of value:

*Diet A*

	gm.
Casein .....	18.0
Starch .....	54.0
Crisco .....	24.0
Salts (Osborne-Mendel) ....	4.0
Dried brewer's yeast (daily) .	0.2

For the production of rickets the Pappenheimer-Zucker Diet D was also used with excellent results.

*Diet D*

	gm.
Patent flour .....	80.9
Egg albumin.....	10.0
Butter fat .....	5.0
Salts .....	4.1

The salt mixture is based upon analyses of the milk of small animals instead of cow's milk as in the Osborne-Mendel mixture. The composition of these salt mixtures is appended for the purpose of comparison.

*Composition of salt mixture of Diet D*

	gm. per 100 gm. diet
KCl .....	0.85
Na <sub>2</sub> CO <sub>3</sub> .....	0.85
MgCO <sub>3</sub> .....	0.286
Ca lactate .....	2.000
Ferric citrate.....	0.100
KI .....	0.0002
MnSO <sub>4</sub> .....	0.00078
NaF .....	0.0024
KAl(SO <sub>4</sub> ) <sub>2</sub> .....	0.00024

*Osborne-Mendel salts*

	gm.
CaCO <sub>3</sub> .....	134.8
MgCO <sub>3</sub> .....	24.2
Na <sub>2</sub> CO <sub>3</sub> .....	34.2
K <sub>2</sub> CO <sub>3</sub> .....	141.3
KI .....	0.02
MnSO <sub>4</sub> .....	0.079
NaF .....	0.248

$K_2Al_2(SO_4)_4$ .....	0.0245
$H_3PO_4$ .....	103.2
HCl .....	53.4
$H_2SO_4$ .....	9.2
Fe citrate+1.5 $H_2O$ .....	6.34
Citric acid+ $H_2O$ .....	111.1

The dietary constituents, with the exception of casein, were used as purchased. The casein was extracted three times, three hours each extraction, under reflux with boiling alcohol. It was then dried at 110°C for 48 hours.

It is manifestly impossible, as well as unnecessary, to record in detail the results obtained with every experimental animal. We shall merely select typical data to illustrate the nature of our findings.

## DISCUSSION

### *Antiophthalmic action of cod liver oil concentrate*

It may be seen from Table I that the alcoholic extract of cod liver oil did not cure ophthalmia, while the residual oil (rat 212) did. It is further evident that the saponified acid extract (rat 217) was effective in curing ophthalmia. Treatment of the cod liver oil concentrate (C-19) with charcoal (rats 184, 199 and 200) or fuller's earth (rat 183) did not destroy its potency.

It is interesting to note that from Nov. 17 to Dec. 18, rats 183 and 184 received a number of fractions which proved to be inactive. During the month there was a loss of 47 gm. and 19 gm. respectively. However, when the active fractions C-20 and C-21 were given there was a gain of 31 gm. and 32 gm. respectively in only 15 days.

In the case of rat 200, a cure was obtained accompanied by loss in weight. The same phenomenon was noticed a number of times; it was due undoubtedly to a decreased consumption of food resulting from some organic disturbance.

Although vitamine A is known as the "growth-promoting, antiophthalmic vitamine", satisfactory growth on a given diet will take place only if it is adequate as regards the quality, quantity and caloric value of its components. Under suitable conditions, any one of the dietary constituents may be the limiting factor in growth. Accordingly, it is essential to exercise care in this respect in the evaluation of any of the vitamins.

An illustration of this is afforded in the varying results obtained by different investigators. Thus, Goldblatt and Zilva<sup>9</sup>

TABLE I.  
*Growth-promoting and antiophthalmic action of cod liver oil concentrates.*

**Fraction	Rat	Initial	Ophthalmia	Weight - gm.		Remarks
				Not cured	Cured	
C - 7	204	Aug. 15, 45	Oct. 14, 81	Nov. 6, 96	Nov. 13, 109	Alcoholic extract of cod liver oil. C - 7 started Oct. 14; C - 8 started Nov. 6.
C - 8	212	Aug. 21, 48	Oct. 14, 82	—	Nov. 12, 125	Residual oil from alcoholic extraction. C - 8 started Oct. 14.
C - 11	218	Aug. 21, 44	Nov. 23, 118	—	Dec. 11, 133	Acid extract of cod liver oil. C - 11 started November 23.
C - 19	217	Aug. 21, 45	Nov. 24, 130	—	Dec. 14, 151	Saponified acid extract. C - 19 started November 24.
C - 20	200	Aug. 14, 46	Dec. 16, 130	—	Jan. 19, 100	C - 19 after treating with charcoal ("norit"). Fraction started Dec. 16.
	199	Aug. 14, 51	Dec. 15, 149	—	Jan. 16, 210	Fraction started Dec. 15.
	184	Aug. 4, 56	Nov. 17, 159*	Dec. 16, 140	Jan. 1, 172	Fraction started Dec. 16.
C - 21	183	Aug. 4, 52	Nov. 17, 179*	Dec. 16, 132	Jan. 1, 163	C - 19 after treating with fuller's earth. Fraction started Dec. 16.

\* From November 17 to December 16, animals received a number of fractions which proved to be inactive.

\*\* All Fractions administered apart from the diet (diet A) in amounts equal to 0.100 gm. cod liver oil daily, together with 0.200 gm. dried brewers' yeast.

found that the minimal growth-promoting dose was 2.2 mg. cod liver oil per day, using rats on a diet composed of



Casein (heated and oxidized)...	20 gm.
Starch .....	50 "
Cotton seed oil (hardened) .....	15 "
Salts (McCollum No. 185) .....	5 "
Lemon juice (decitrated) .....	5 cc.
Marmite .....	5 "
Water (distilled) .....	50 "

*Composition of salts No. 185*

NaCl .....	51.9 gm.
MgSO <sub>4</sub> (anhydrous) .....	164.0 "
NaH <sub>2</sub> PO <sub>4</sub> + H <sub>2</sub> O .....	194.1 "
K <sub>2</sub> HPO <sub>4</sub> .....	286.2 "
Ca (H <sub>2</sub> PO <sub>4</sub> ) <sub>2</sub> + H <sub>2</sub> O .....	162.0 "
Calcium lactate .....	390.0 "
Ferric citrate .....	35.4 "

Substantiating this, there is the work of Holmes<sup>10</sup> who observed that as little as 1.0 mg. cod liver oil per day sufficed to promote growth and to cure ophthalmia in rats on a diet of

Casein .....	18 gm.
Cornstarch .....	28 "
Milk sugar .....	28 "
Cotton seed oil (or peanut oil) .	22 "
Salts (Osborne-Mendel) .....	4 "
Dried brewer's yeast (daily) ..	0.2 "

Our own investigation also confirms the above, using Diet A, as the data in Table II will show. While the animals as a whole did not grow as well as they might have, still in the experimental

TABLE II.

*Growth-promoting and antiophthalmic action of cod liver oil concentrate.*

Fraction	Rats	Average weight, gm.			Gain	Remarks
		Jan. 26	*April 9	May 17		
Control .....	668 - 670	56	119	135	16	#668 Ophthalmia, May 7. #670 Ophthalmia, May 14.
**Cod liver oil.....	660 - 662	56	121	150	38	No ophthalmia.
***Concentrate .... C - 37	656 - 658	51	117	149	32	No ophthalmia.

\* Fractions started; given apart from diet (diet A) mixed with daily portion of 0.200 gm. dried brewers' yeast.

\*\* 0.0036 gm. Cod liver oil daily.

\*\*\* Similar to C - 19 (Table I) Gave 0.0000039 gm. Concentrate daily, corresponding to 0.0036 gm. Cod liver oil.

period, those rats receiving cod liver oil or cod liver oil concentrate gained twice as much weight as the controls.

Somewhat different results were obtained by Steenbock, Jones and Hart,<sup>4</sup> who showed that a level of 0.1 and 0.5 per cent. cod liver oil, or its ether-soluble non-saponifiable portion, did not suffice to promote growth or to prevent ophthalmia. At a level of 1 and 2 per cent., all of the rats but one remained normal. The ether-soluble non-saponifiable fraction was therefore just as active as the fresh cod liver oil from which it was made, gram for gram. Based upon an average food intake of 7.5 gm. per day, it appears that the rats required at least 75 mg. cod liver oil per day for normal nutrition. In this work the diet consisted of

Casein (extracted) .....	18 gm.
Salts (No. 32) .....	4 "
Agar .....	2 "
Yeast .....	2 "
Dextrin .....	74 "

*Composition of salts No. 32*

NaCl .....	0.202 gm.
MgSO <sub>4</sub> (anhydrous) .....	0.311 "
Na <sub>2</sub> HPO <sub>4</sub> + 12H <sub>2</sub> O .....	0.526 "
K <sub>2</sub> HPO <sub>4</sub> .....	1.115 "
Ca <sub>2</sub> H <sub>2</sub> (PO <sub>4</sub> ) <sub>2</sub> + 4H <sub>2</sub> O .....	1.116 "
Calcium lactate .....	0.289 "
Ferric citrate .....	0.138 "

It may be that the greater need for cod liver oil in these experiments was due to the high carbohydrate, low fat diet used. That this is possible is apparent from our experiments showing that the vitamine B requirements of rats vary with the composition of the diet.<sup>11</sup> This has been demonstrated as well by a number of other investigators.

From the foregoing data, it is evident that one must be cautious in drawing conclusions as to the growth-promoting, antiophthalmic action of any particular substance under investigation. This is further emphasized by the fact that we have occasionally had cases of sore eyes which could be cleared up merely by washing with boric acid. In direct opposition to this, we have seen instances of ophthalmia which did not yield to treatment even

with cod liver oil, known to have cured other cases. Presumably, we have here a type of ophthalmia similar to that reported by McCollum, Simmonds and Becker<sup>12</sup> as being due to some disturbed relationship in the mineral content of the diet. It is important, therefore, in studying the growth-promoting antiophthalmic action of a substance, to make certain that it is actually the limiting factor.

*Antirachitic action of cod liver oil concentrate*

The radiographs and microscopic sections are self-explanatory and show that the cod liver oil concentrate manifests the antirachitic effect characteristic of fresh cod liver oil. As for quantitative results, there is the general impression that 0.100 gm. cod liver oil daily is sufficient to protect against or to cure rickets. Accordingly in the experiments illustrated, we used sufficient concentrate to correspond to 0.100 gm. cod liver oil daily.

As regards the ether-soluble, non-saponifiable portion of cod liver oil, there is ample evidence establishing that it is equally as effective as fresh cod liver oil in its antirachitic action. Here too, as in the case of antiophthalmic action, it is essential to be certain that the substance being tested for its antirachitic value is in effect the limiting factor.

We have inaugurated quantitative experiments to establish the minimal antirachitic dose of cod liver oil and of cod liver oil concentrate. The results will appear in an early publication.

### SUMMARY

By means of an acid extraction followed by saponification, obviating the necessity of handling large quantities of material, we have prepared a concentrate from cod liver oil which in the crude state is 2000 times more active than fresh cod liver oil, both as regards the antirachitic and antiophthalmic vitamins.

Thus, 1000 gm. fresh cod liver oil yields 0.5 gm. crude active concentrate — a brown syrupy mass which, on standing, crystallizes in light yellowish-brown needle-like crystals, radiating from a central point.

On eliminating cholesterol, the 0.5 gm. concentrate is reduced to 0.1 gm., a concentration of 1 in 10,000. Working with great care, it is possible to obtain a concentration of 1 in 15,000.



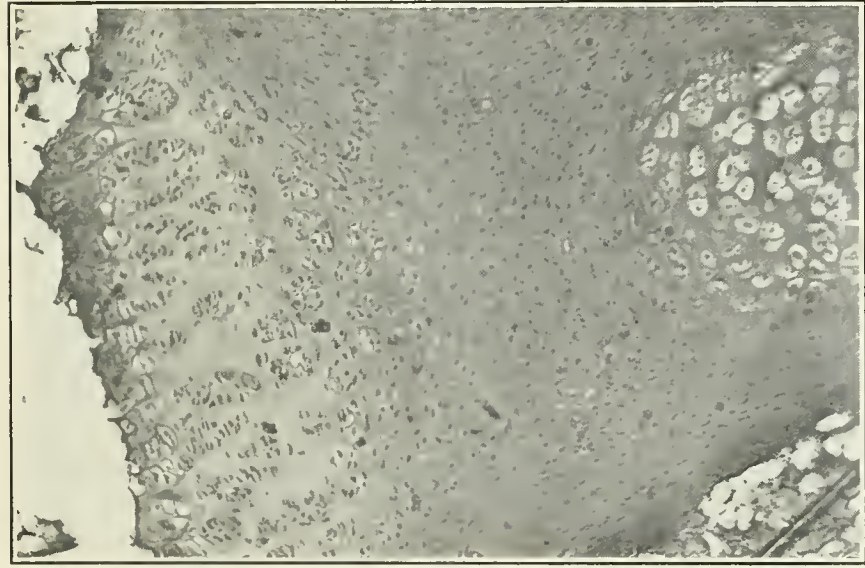


FIG. 1

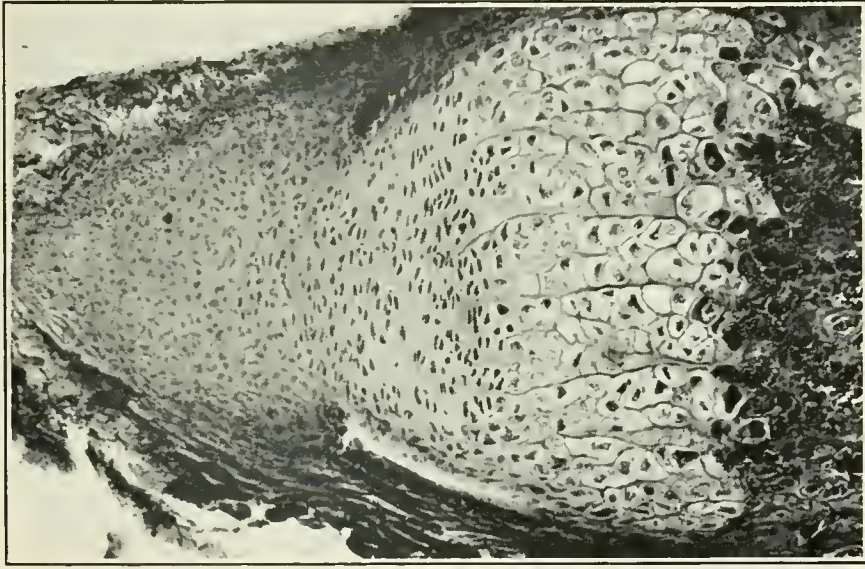


FIG. 3

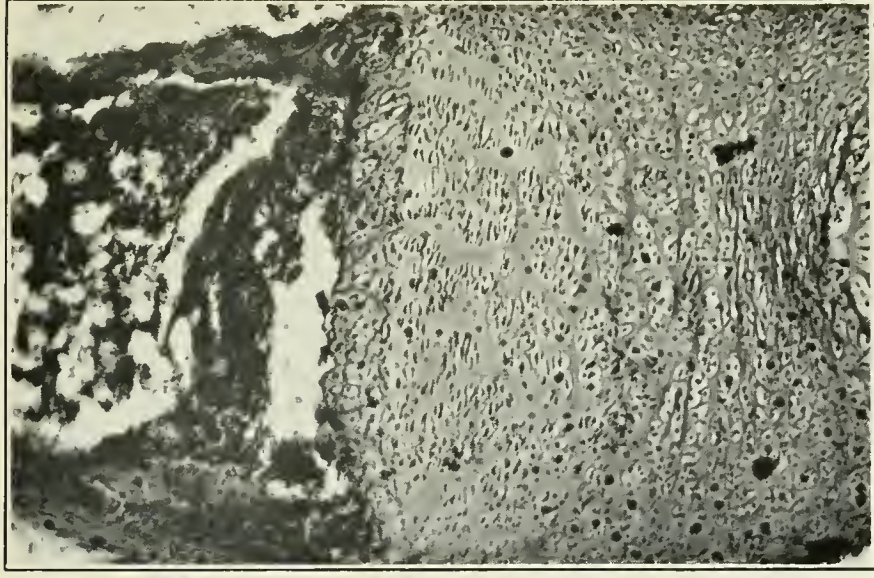


FIG. 5



FIG. 2



FIG. 4



FIG. 6







FIG. 7

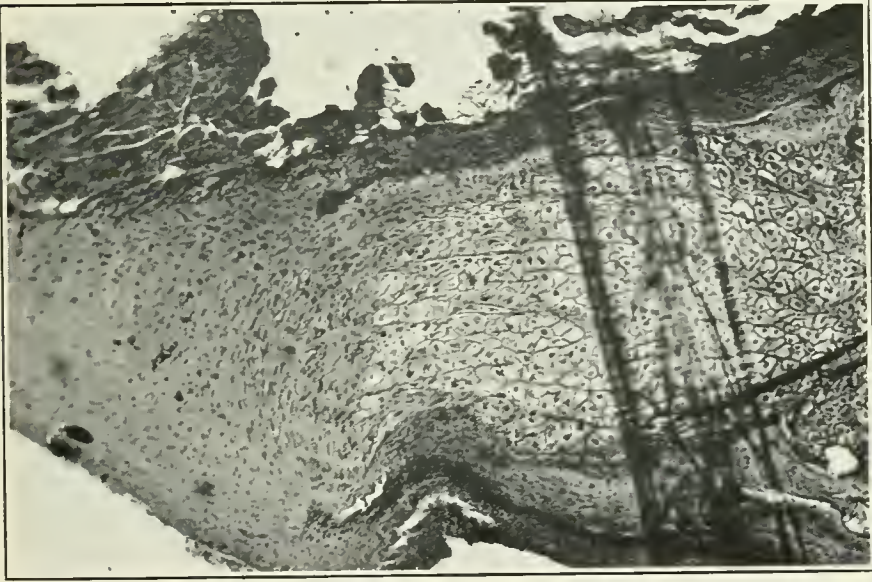


FIG. 9

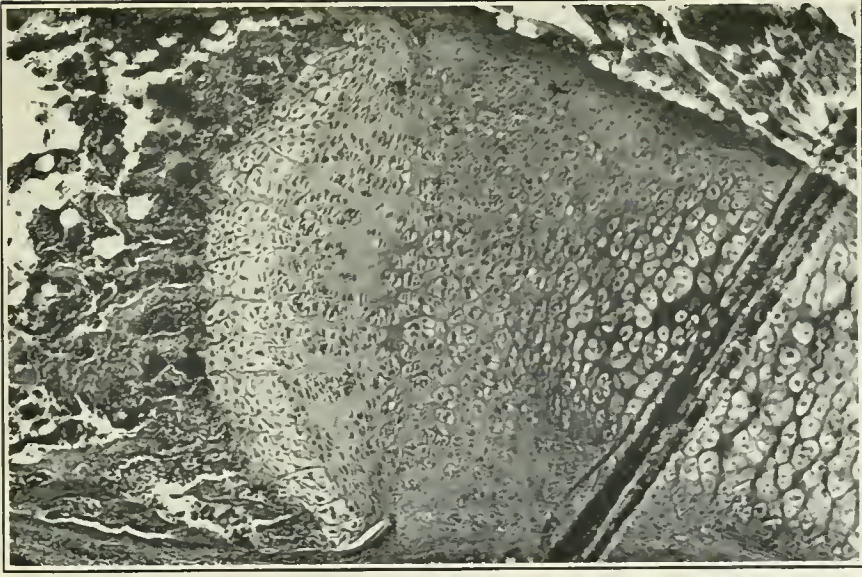


FIG. 11



FIG. 8



FIG. 10



FIG. 12





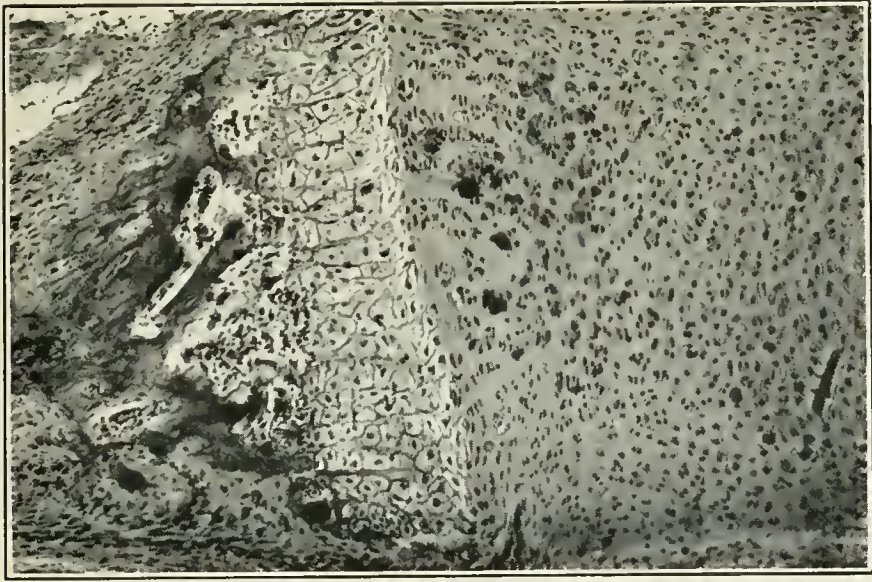


FIG. 17

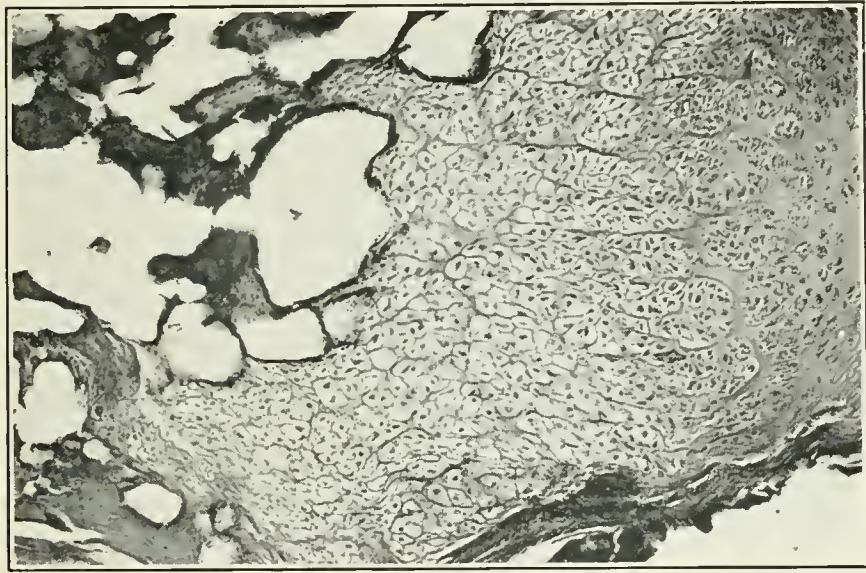


FIG. 15

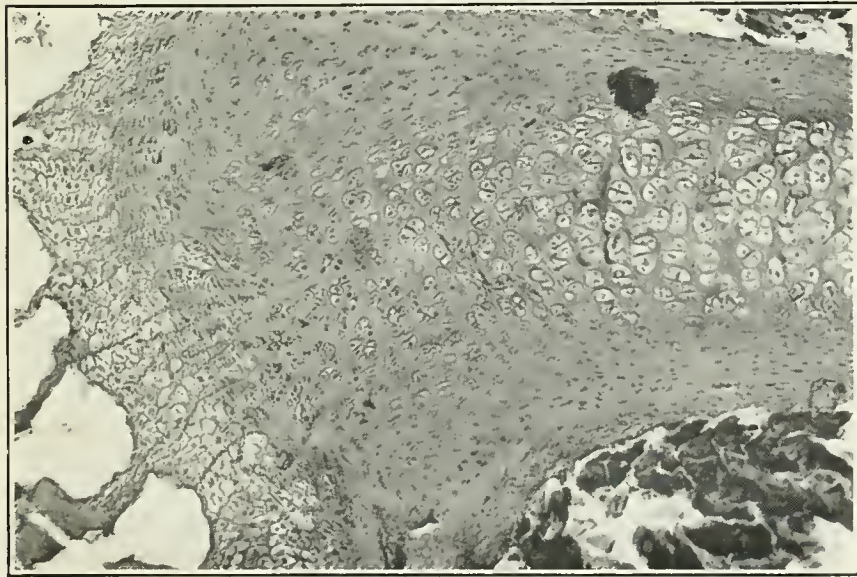


FIG. 13

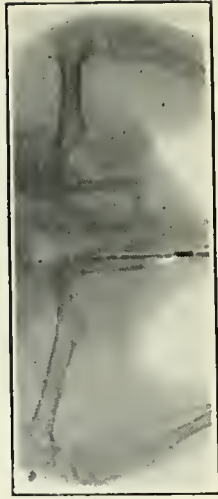


FIG. 18

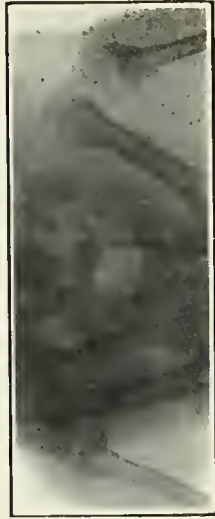


FIG. 16

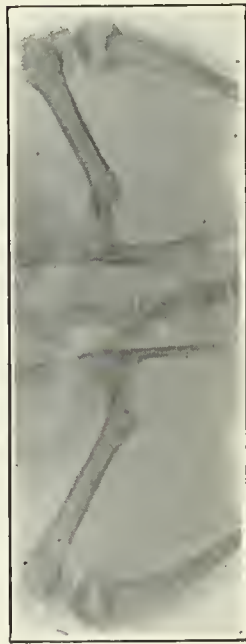


FIG. 14





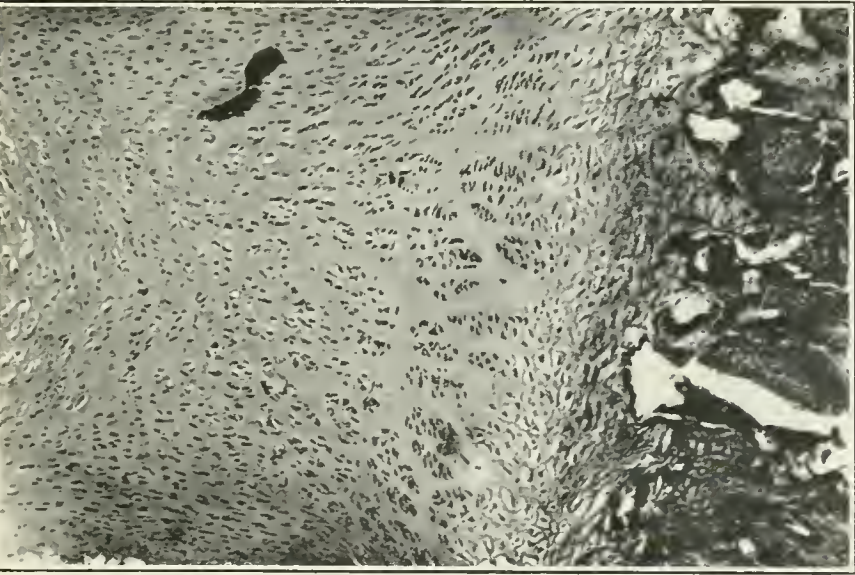


FIG. 19

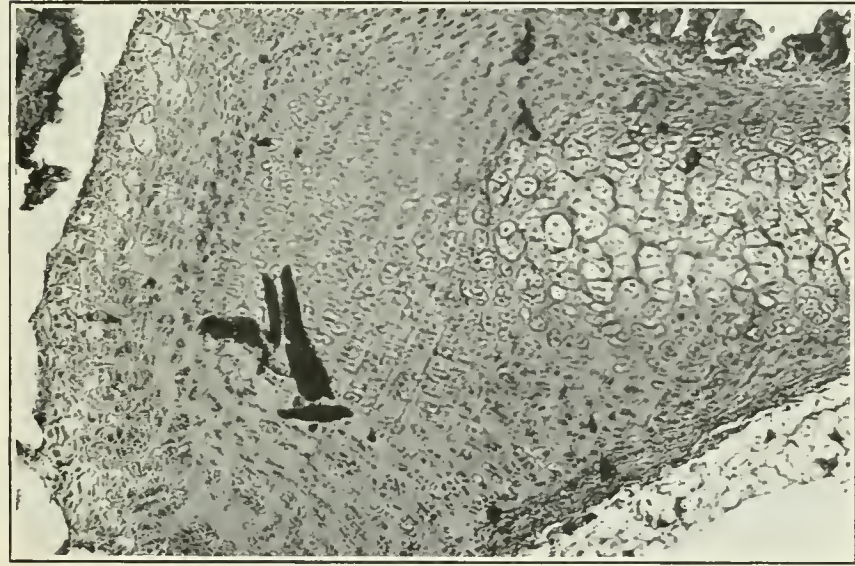


FIG. 21

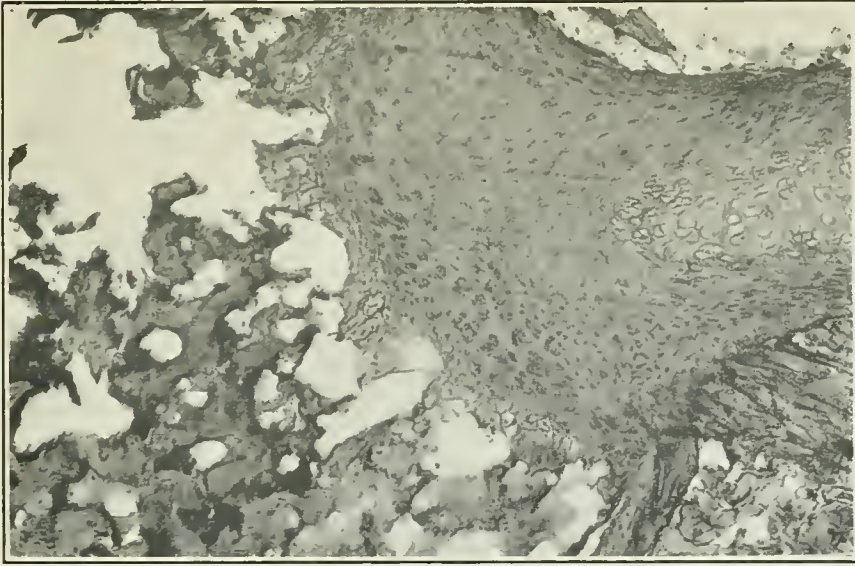


FIG. 23



FIG. 20



FIG. 22



FIG. 24





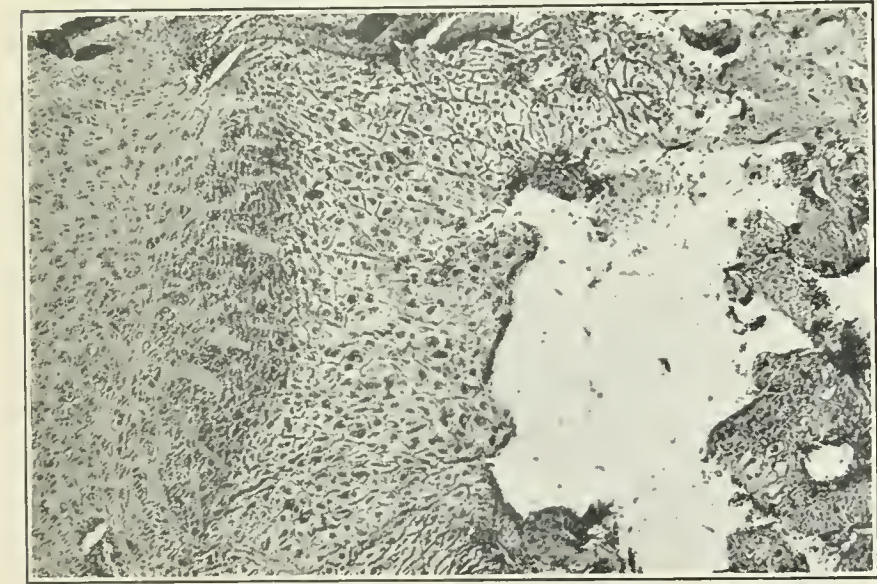


FIG. 25

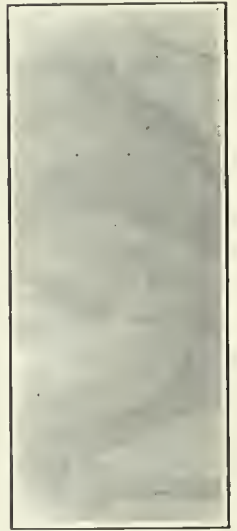


FIG. 26

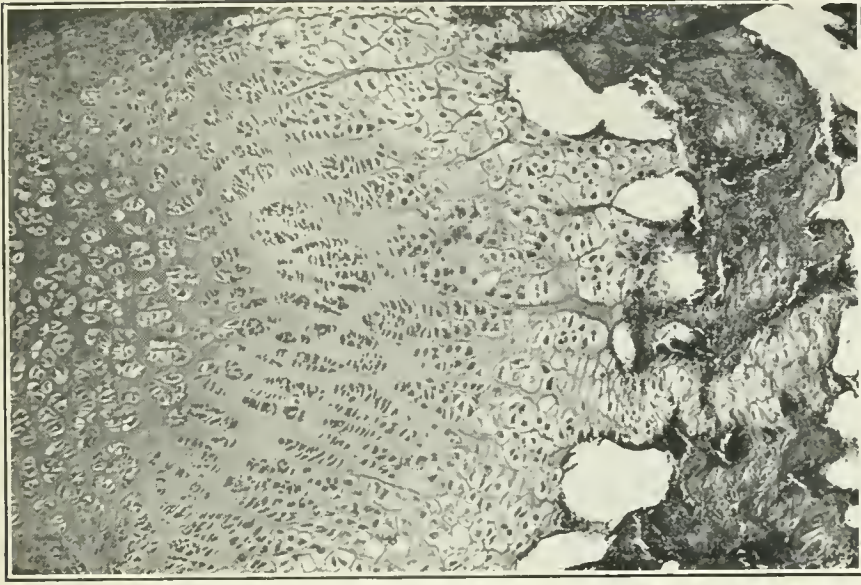


FIG. 27



FIG. 28





The concentrate serves as a convenient starting material for chemical work directed to the actual isolation and identification of the vitamins present in cod liver oil.

Higher concentrations may be obtained by distillation with superheated steam. The active fraction may also be distilled at about 250°C in a vacuum of about 2 mm.

Although the chemical composition of the concentrate has not been established as yet, the presence of C, H and O has been demonstrated. N, S and P are absent, as are also the halogens. The  $\text{H}_2\text{SO}_4$  reaction is obtained just as with fresh cod liver oil.

The concentrate may be hydrogenated without destroying its activity.

Neither charcoal nor fuller's earth will adsorb the concentrate from an ether solution.

The concentrate is not toxic even in very large doses. It may be mixed with sugar and compressed into 1 grain tablets equivalent to practically any desired dosage of cod liver oil. This offers an effective method of cod liver oil therapy, whether experimental or practical.

Clinical experiments have shown that the concentrate is as effective in children as in animals.

Our observations lead us to believe that infants, as early as one month after birth, should be given cod liver oil if it can be tolerated; if not, the child should receive the equivalent of cod liver oil in the form of a concentrate, as a prophylactic against rickets and other nutritive disturbances traceable to the lack of fat-soluble vitamins in the diet.

The adoption of prophylactic treatment will eventually mean the elimination of rickets as a factor in infant nutrition.

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### EXPLANATION OF FIGURES

All fractions administered daily from the beginning of the experiment (except where otherwise indicated) in amounts corresponding to 0.100 gm. fresh cod liver oil. Radiographs made at the termination of the experiment.

Fig. 1. Rat. 325. Diet D for 30 days. Fraction C - 6 (fresh cod liver oil). Rib shows no rickets.

Fig. 2. Rat. 325. Radiograph showing no rickets.

Fig. 3. Rat 390. Diet D for 30 days. Fraction C - 6. Rib showing no rickets.

Fig. 4. Rat 390. Radiograph shows no rickets.

Fig. 5. Rat 315. Diet D for 42 days. Fraction C - 11 (acid extract of cod liver oil) started on the 8th day. Rib shows no rickets.

Fig. 6. Rat. 315. Radiograph shows no rickets.

Fig. 7. Rat 343. Diet D with substitution of 18% Crisco for 22 days, followed by Diet D for 28 days. Fraction C - 12 (residual oil after acid extraction). Rib shows typical rachitic changes.

Fig. 8. Rat 343. Radiograph shows rickets.

Fig. 9. Rat 613. Diet S. R. 4. for 36 days followed by Diet D for 32 days. Fraction C - 36 (residual oil after acid extraction) started on 36th day. Rib shows rickets.

Fig. 10. Rat 613. Radiograph showing rickets.

Fig. 11. Rat 319. Diet D for 33 days. Fraction C - 19 (ether soluble substance obtained from saponified acid extract of cod liver oil). Rib shows no rickets.

Fig. 12. Rat 319. Radiograph showing no rickets.

Fig. 13. Rat 585. Diet S. R. 4. for 17 days followed by Diet D for 45 days. Fraction C - 29 (ether soluble substance obtained from saponified acid extract of cod liver oil) given from the 1st to the 17th day; omitted from the 17th to the 42nd day; again given from the 42nd day to the end of the experiment. Rib shows healing rickets.

Fig. 14. Rat 585. Radiograph shows healing rickets.

Fig. 15. Rat 336. Diet D with substitution of 18% Crisco for 22 days, followed by Diet D for 28 days. Fraction C - 10 (fatty acids obtained by acidifying the saponification liquor after the latter has been extracted with ether). Rib shows rickets, indicating absence of vitamins in fatty acid fraction.

Fig. 16. Rat 336. Radiograph shows rickets.



Fig. 17. Rat 409. Diet D for 40 days. Fraction C - 20 (fraction C - 19 after treating with charcoal). Rib shows no rickets, indicating that charcoal does not adsorb the antirachitic vitamine.

Fig. 18. Rat 409. Radiograph shows no rickets.

Fig. 19. Rat 416. Diet D for 40 days. Fraction C - 21 (fraction C - 19 after treating with fuller's earth). Rib shows no rickets, indicating that the antirachitic vitamine is not adsorbed.

Fig. 20. Rat 416. Radiograph shows no rickets.

Fig. 21. Rat 575. Diet S. R. 4. for 17 days followed by Diet D for 45 days. Fraction C - 31 (fraction C - 29 after removal of cholesterol) given from the 1st to the 17th day, omitted from the 17th to the 42nd day; again given from the 42nd day to the end of the experiment. Rib shows healing rickets.

Fig. 22. Rat 575. Radiograph shows healing rickets.

Fig. 23. Rat 581. Dietary conditions as in Rat 575. Fraction C - 32 (fraction C - 29 subjected to hydrogenation) given as in Rat 575. Rib shows healing rickets. The substance is therefore stable to hydrogenation.

Fig. 24. Rat 581. Radiograph shows healing rickets.

Fig. 25. Rat 572. Dietary conditions as in Rat 575. Control animal. Rib shows rickets.

Fig. 26. Rat 572. Radiograph shows rickets.

Fig. 27. Rat 326. Diet D for 30 days. Control animal. Rib shows typical rickets.

Fig. 28. Rat 326. Radiograph showing rickets.



## A STUDY OF CLINICAL RICKETS

COMPARISON OF RESULTS OBTAINED ON EXPOSURE TO SUNLIGHT  
AND ON TREATMENT WITH COD LIVER OIL OR AN ACTIVE  
CONCENTRATE PREPARED FROM COD LIVER OIL.

BY LOUIS FISCHER, M. D.

(From the Infantorium and Nursery of the Heckscher Foundation,  
New York City)\*

It is only within the last few years that the treatment of rickets has been placed upon a definite scientific foundation by the proof that cod liver oil is a specific cure for rickets. This disease ordinarily develops during the first seven months of life. Children born in the Autumn usually develop rickets in the Spring following. This seasonal incidence was well established in the studies made in Vienna by the Medical Research Council. In this connection, von Hansemann pointed out that almost all children born during the late part of the year and dying the following Spring showed evidence of rickets. Those born in the Spring and dying in the Autumn are free from rickets.

We have been interested in this important problem for many years. In a large class of undernourished infants sent to our Institution by the various Social Services of the city hospitals, by milk stations of the Board of Health and by Babies' Welfare organizations, delayed dentition, craniotabes and rachitic manifestations in the thorax and spine were noted. Many of these children, although from 15 to 24 months old, were unable to stand.

Our observations have been carried on since 1918. Out of the total of 900 infants examined, opportunity was afforded for a most careful scrutiny of 200 cases. Of these, 37 showed clinical evidence of rickets; this was confirmed by the radiograph. A number of these babies were prematurely born and several were

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\* A preliminary communication was made before the Soc. Exp. Biol. and Med., May 24, 1924, at Yale University.



raised in incubators. Of the 37 rickets cases, 10 were syphilitic.\*

Regarding food as a causative factor, nothing definite can be stated. Many of the infants were partially breast-fed while some were entirely breast-fed. In the latter group, severe rickets was noted in one child 18 months, another 17 months and a third 15 months old. These cases were nursed irregularly and without a definite feeding interval, night or day. Undoubtedly we are dealing here with a deficiency in both the quality and quantity of the mother's milk. It is obvious therefore that human milk, like cow's milk, may sometimes be included in the etiology of rickets.

Some of the cases under investigation had lived in congested districts while others had been in large hospitals. It was noted that the out-of-door child exposed to sunlight and fresh air was not always free from rickets. Thus, several infants living under modern hygienic conditions, but improperly fed, developed rickets.

Many of these children were artificially fed and hence were properly classed as deficiency cases. Most of these had received cow's milk largely diluted with water. As a result, the energy quotient was often less than 50 calories per kilo. In such cases, loose bowels, greenish stools and a large quantity of mucus was observed, together with considerable weight disturbance. As a rule, these babies responded to proper nutrition and most of them improved on a starvation diet of tea for one day, followed by Finkelstein's protein-milk.

Many of the infants showed enlarged costo-chondral junctions; despite this, the bony structures and epiphyses of the arms and legs did not show a typical rickets by means of X-ray.\*\* Cranio-tabes is a very early and important symptom. Some of the cases developed rickets without our being able to elicit the determining factor.

In several instances, the stool was normal and the food properly metabolized. There was also a steady gain in weight and to all appearances the babies seemed to thrive. Dentition appeared about the sixth month in one case; in another, at the

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\* We are indebted to Dr. John A. Fordyce for valuable aid in cases of luetic suspicion and for therapeutic suggestions; also to his laboratory for courtesies in examining spinal fluid and blood for Wassermann reactions.

\*\* We are indebted to Dr. Charles Gottlieb for most of the X-ray work and for able assistance in the early diagnosis of rickets.

seventh month. Nevertheless, radiographic examination showed evidence of rickets. Evidently, an early eruption of a tooth at six months, while appearing to show excellent development, may serve to obscure a latent rickets, otherwise detectable by the X-ray.

A surprisingly large number of cases of rickets were referred to us as "difficult feeding cases." Four had multiple furunculosis; three showed tuberculosis in some form — glandular, pulmonary, or meningeal; one suffered with gastro-intestinal disturbances caused by congenital syphilis; six were hypertonic cases with pyloric spasm.

The latter were very difficult to handle and did not respond satisfactorily, owing largely to continuous vomiting, with consequent loss of food. In this connection, the literature reports a number of cases benefitted by the use of thick cereal feeding. Giving 1 or 2 teaspoons of farina, so thick that it cannot be poured, before each feeding seems to aid in retaining the milk feedings. Thin cereals, are not so advantageous.

Structural changes of a pathological nature, backwardness in development, delayed dentition, and frequently craniotabes were prominent symptoms showing the presence of rickets. Early substantiation of the diagnosis was made possible by means of the X-ray.

Radiographs taken at frequent intervals indicated admirably the therapeutic effect of the treatment instituted. The importance of the X-ray in rickets cannot be overestimated. It gives a clear picture of progress which formerly could only be imagined.

In a number of cases, including some with pyloric spasm where gastric irritability existed and where fresh cod liver oil seemed to irritate the gastric mucosa, we have made use of an active concentrate\* prepared from cod liver oil. This concentrate contains the vitamins originally present in fresh cod liver oil, is non-toxic even in large doses and is easily assimilated. This is a therapeutic advance and one which we expect to make use of on a larger scale in continuing our work.

The cod liver oil concentrate was first administered mixed with Karo syrup. It was subsequently found more convenient to give

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\* This cod liver oil concentrate is similar to that prepared and used by Dubin and Funk in their studies on experimental rickets, the results of which appear in this issue.

a 1 grain tablet in which the cod liver oil concentrate is mixed with sugar, so prepared that each tablet is the equivalent of a half teaspoon of fresh cod liver oil. To insure against possible loss of activity due to oxidation, the tablets are coated.

The tablets are best administered by crushing and dissolving in a teaspoon with a little water, milk, or orange juice. In this way one is certain that the substance has been completely taken. The dosage is the same as for fresh cod liver oil.

In order to conserve space, only six typical cases are described in detail. The radiographs are indicative of the progress made.

*Case 1.* — Allan R. Twin; admitted to Infantorium in February, 1924. Age 1 year 6 weeks. Breast-fed 11 months, then received breast and bottle; after 1 year received cereal, potato and broth. Formula for bottle: Grade A milk 7 ounces, water 1 ounce; 5 feedings. Very slow in development. Mother noticed he did not attempt to stand, and was not as bright as twin. Twin very well, but pale. Mother's and father's Wassermann reported negative by Board of Health.

History: Cannot stand, cannot walk. Shows evidence of rickets in radius, ulna, tibia, fibula and also thorax. Has secondary anemia. Blood examination showed: Red cells 3,100,000, White cells 9,500, hemoglobin 50 per cent. Case diagnosed as rickets. X-ray examination made on Feb. 23, 1924, shows cupping at lower end of each radius and ulna. Findings confirm the clinical diagnosis of rickets.

The child was placed on fresh cod liver oil,  $\frac{1}{2}$  teaspoon three times a day. Egg-yolk, milk and vegetable, chiefly spinach, were given. Daily exposure to sunlight was also provided for.

The second radiographic examination, March 10, showed spreading of the lower end of radius and ulna. Condition considerably improved since last examination.

*Case 2.* — Helen L. 6 months old; bottle baby. Was born in California amid sunshine and fresh air. Its hygienic supervision was of the best. The infant's feeding was supervised by a well-known pediatricist, and the metabolism as far as the history is concerned was perfect. The bowels were inclined to constipation. The infant weighed  $5\frac{1}{2}$  pounds at birth and gained steadily although the gains were not very large. Two symptoms were noted — profuse perspiration, and restlessness at night. At 6 months the infant weighed about 10 pounds, the muscles were flabby, and there was marked craniotabes. The X-ray examination showed pronounced cupping of the ulna and radius. We also noted a scoliosis. Weekly X-ray examinations were made to study the progress of the calcium deposit in the bones.

Some improvement followed the administration of cereals and vegetables during the first month; exposure to sunshine was resorted to when possible. The progress noted was gain in weight, better color, less restlessness, and better appetite. Other clinical progress was the sudden eruption of teeth, and the ability to hold the head. The kyphosis improved but slightly. The



X-ray, however, disappointed us after one month's treatment, very little change in the bones being noted. Cod liver oil concentrate was then ordered, in the form of a tablet containing the equivalent of  $\frac{1}{2}$  teaspoon of cod liver oil, three times a day. Rapid progress of the calcium deposit and healing of rickets within one month of treatment were observed.

*Case 3.*—Cato M. Age 17 months. Was admitted because of inability to stand or walk. Crackling râles heard at the right base posterior; marked dyspnea at times. Has a large pendulous belly and screams night and day. Admission diagnosis; inanition, T. B. C. and probable meningitis. Lumbar puncture was performed, 30 cc. of clear fluid withdrawn; no T. B. bacilli or other organisms found. Spinal fluid reported normal. X-ray of the chest showed enlarged bronchial glands; no evidence of consolidation or T. B. C. Radiographic examination showed an advanced degree of rickets, as indicated by marked cupping of lower ends of tibia and fibula, and lower ends of radius and ulna. There is evidence of old fractures through the right radius and ulna, and through the left fibula. Diagnosis: rickets, kyphosis.

The treatment first consisted of exposure to the mercury vapor quartz lamp for four minutes on the first day. Later, the child was exposed 5, 6 and up to 10 minutes daily or every two days, depending on the amount of skin tolerance. Some infants burn easily and show erythema lasting one or more days. This treatment did not result in specific healing of rickets.

Following the administration of one tablet of cod liver oil concentrate daily for a period of two weeks, the radiograph showed a decided deposition of calcium. At the end of three weeks, healing had progressed very rapidly.

*Case 4.*—John W. 5 months old; weight, 8 pounds, 8 ounces. Mother died of Bright's disease, following convulsions and edema; was in uremia 4 days. Infant at birth weighed 7 pounds, 4 ounces; gained but 1 pound, 4 ounces, in 5 months. It had received breast milk from wet nurse, later condensed milk and water. The infant vomited frequently. The extremities had a spastic rigidity, the bowels were inclined to constipation and this alternated with loose greenish mucous stools. The appetite was poor and still the infant had its hands in its mouth most of the time. There were mucous râles on both sides of chest. Radiographic examinations excluded tuberculosis of the lungs. The extremities showed thickening at the lower ends of the radius and ulna, and thickening at the bases of the phalanges. Diagnosis: rickets, bronchitis, and marked inanition. This is a hypertonic baby. Many modifications of food were made but without avail. Finally human milk was given. In two months from the time that treatment was commenced, the child had gained over 4 pounds and weighed 12 pounds, 10 ounces. Radiographic examination showed a slight improvement in the bony structure, but there was no specific healing effect on rickets. At the age of 10 months the child weighed 14 pounds, 6 ounces. Though the bones were stronger, the muscles firmer and general nutritive condition improved, there was still no evidence of dentition.

Owing to the hypersensitiveness of the gastric mucosa the child could not tolerate egg-yolk, spinach pulp or carrot pulp; nor was fresh cod liver oil retained. As a result, radiographic examination showed no progress.

A half tablet of the cod liver oil concentrate, equal to one quarter teaspoon of fresh cod liver oil was then given progressively — three, four and five times a day, after each feeding. In the end the child was receiving the equivalent of  $1\frac{1}{4}$  teaspoons fresh cod liver oil daily. The concentrate was easily tolerated and its effectiveness was amply demonstrated by the radiograph.

*Case 5.* — Anna H. 10 months old. Was admitted at the Infantorium with history of backwardness in sitting, no teeth, unable to support head and spine, profuse perspiration and slight injury to the head. Child admitted to St. Luke's hospital with a sub-periosteal hemorrhage; cried most of the time as though in pain, and was very restless. Clinical diagnosis: rickets.

Radiographic examination shows irregular worm-eaten lower ends of the right and left radius and ulna, and right and left tibia and fibula. There is no evidence of scurvy. This case is typical of the severer form of rickets encountered.

Egg-yolk and spinach juice were added to the diet. In addition, the child was exposed to sunlight. A gradual improvement was noted but specific deposition of calcium and healing took place only when fresh cod liver oil was administered.

*Case 6.* — Baby G. Age, 11 months; has been suffering with constipation, anorexia and insomnia; restless by day; and seems dissatisfied. There was marked craniotabes and also beaded ribs. The epiphyses of the ulna and the radius were large and spongy. Clinical diagnosis: severe rickets. Radiographic examination confirms diagnosis.

The quartz lamp therapy was tried with a radical change of diet in which cereals, vegetables, eggs and milk formed the chief bulk of food. No definite improvement was noted by the X-ray until cod liver oil concentrate was given. Four weeks later the rachitic manifestations were practically undiscernible. Shortly thereafter we noticed the sudden eruption of four teeth; the craniotabes became less marked; the appetite improved and constipation disappeared.

A review of all of our cases leads to the conviction that exposure to sunshine or to the mercury vapor quartz lamp is of some benefit in the treatment of rickets. The same is likewise true of egg-yolk feeding. Our findings thus confirm those of a number of other investigators.

Nevertheless experience has proved that even under the most favorable conditions absolute reliance cannot be placed upon proper hygiene and food as a protection against rickets. In the final analysis, we must recognize the importance of another factor — cod liver oil — which is a proven specific in the prevention and cure of rickets.



That the action of cod liver oil is due to its vitamine content is no longer a matter of controversy. It has been shown that when cod liver oil, or an organic acid extract of cod liver oil, is saponified and extracted with ether, a highly concentrated semi-crystalline substance, containing the antirachitic and antiophthalmic vitamins present in fresh cod liver oil is obtained. That is, the vitamins of cod liver oil are found in the ether-soluble, non-saponifiable portion of the oil.

This vitamine fraction, when mixed with sugar and compressed into tablets, furnishes a practical method of applying cod liver oil therapy, particularly in cases where fresh cod liver oil is not tolerated.

Admittedly, a combination of proper hygiene, a well-balanced diet and cod liver oil, or the active substance present in the cod liver oil, is the best means of combating rickets. Its greatest use lies in the field of prophylaxis. In this respect it is well to keep in mind the fact that rickets not infrequently prevails as well in breast-fed as in bottle-fed babies. Prophylaxis should therefore also include this class of children.

We do not doubt that concerted action along these lines would eventually make rickets an exceedingly rare occurrence.

#### SUMMARY

During the course of six years, we have had occasion to examine 900 children sent to our Institution for treatment. A detailed and careful examination of 200 of these revealed 37 cases of rickets in varying degrees of severity. After suitable regulation of the diet, they were divided into three groups. The first was subjected to the action of sunlight. The second was given fresh cod liver oil. The third received an active concentrate prepared from cod liver oil.

Progress was controlled by frequent radiographic examinations, in addition to clinical observations. Typical healing was noted in all cases, although the most marked improvement was evident in those children receiving cod liver oil or cod liver oil concentrate in the form of 1 grain tablets. The latter are well tolerated, easily assimilated and as effective therapeutically as fresh cod liver oil.



## CONCLUSION

As a result of our investigation we are of the opinion that in the management of rickets, every known therapeutic agent should be made use of. The best results are obtained under a treatment combining proper food, cod liver oil and exposure to sunlight.

In the numerous cases where fresh cod liver oil is not tolerated, an active concentrate prepared from cod liver oil, and free from the latter's disagreeable and objectionable features, may be used with success.

We earnestly recommend that cod liver oil therapy, whether one uses fresh cod liver oil or a cod liver oil concentrate, should be regarded as a prophylactic as well as a curative measure.

If prophylactic treatment were universally instituted in all children, even as early as one month of age, rickets would in due course of time be a thing of the past.

## DESCRIPTION OF PLATES

Case 1. Fig. 1. Rickets, Cupping and fraying at lower end of radius and ulna. Given fresh cod liver oil,  $\frac{1}{2}$  teaspoonful 3 times daily.

Case 1. Fig. 2. 7 days later. Fraying less marked.

Case 1. Fig. 3. 21 days later. Fraying and cupping disappeared. Dense deposition of calcium. Bones practically normal.

Case 2. Fig. 1. Rickets. Radius and ulna show cupping and fraying at lower ends.

Case 2. Fig. 2. Slight improvement in tibia and fibula after one month of dietary treatment combined with exposure to sunlight.

Case 2. Fig. 3. Radius and ulna appear normal after 1 month of treatment with cod liver oil concentrate, 1 tablet (equal to  $\frac{1}{2}$  teaspoonful fresh cod liver oil) three times daily.

Case 3. Fig. 1. Severe rickets. Cupping and fraying of distal ends of tibia and fibula.

Case 3. Fig. 2. Slight improvement after 2 weeks exposure to mercury vapor quartz lamp.

Case 3. Fig. 3. Rapid healing after 3 weeks on cod liver oil concentrate, 1 tablet (equal to  $\frac{1}{2}$  teaspoonful fresh cod liver oil daily).

Case 4. Fig. 1. Rachitic condition as indicated by ragged metaphyseal margins.

Case 4. Fig. 2. After 3 weeks on cod liver oil concentrate. Note fine dark lines running across the ends of the bones, showing deposition of calcium.

Case 4. Figure 3. After 5 weeks, bones appear practically normal. Starting with  $\frac{1}{2}$  tablet (equal to  $\frac{1}{4}$  teaspoonful fresh cod liver oil, the dosage was gradually increased till child was getting the equivalent of  $1\frac{1}{4}$  teaspoonful fresh cod liver oil daily).

Case. 5. Fig. 1. Frayed metaphyseal margins of the tibia and fibula shows rickets; indistinct center of ossification.

Case 5. Fig. 2. Bones appear normal after 5 weeks treatment with fresh cod liver oil ( $\frac{1}{2}$  teaspoonful 3 times daily).

Case 6. Fig. 1. Marked rickets. Cupping and fraying rather extensive.

Case 6. Fig. 2. After 5 weeks on cod liver oil concentrate, 1 tablet (equal to  $\frac{1}{2}$  teaspoonful fresh cod liver oil) 3 times daily. Bones look practically normal.

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490<sup>a</sup>



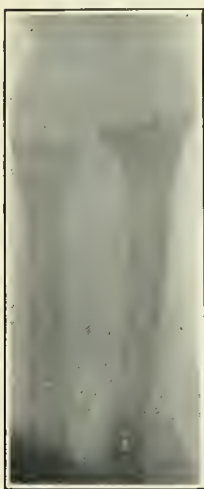
CASE 1—FIG. 1



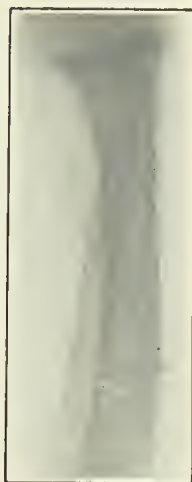
CASE 1—FIG. 2



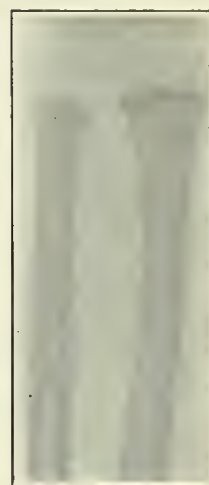
CASE 1—FIG. 3



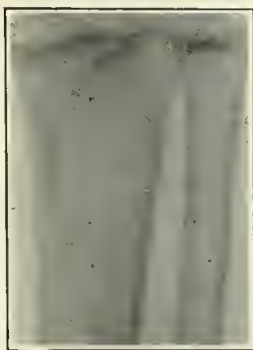
CASE 2—FIG. 1



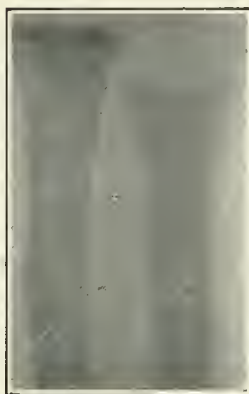
CASE 2—FIG. 2



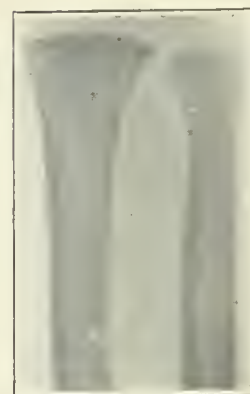
CASE 2—FIG. 3



CASE 3—FIG. 1

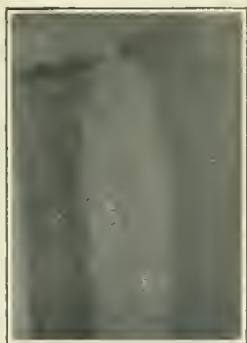


CASE 3—FIG. 2



CASE 3—FIG. 3





CASE 4—FIG. 1



CASE 4—FIG. 2



CASE 4—FIG. 3



CASE 5—FIG. 2



CASE 5—FIG. 1



CASE 6—FIG. 1



CASE 6—FIG. 2





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## FEEDING EXPERIMENTS ON RATS WITH PLANTS AT DIFFERENT STAGES OF DEVELOPMENT.

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In a previous paper<sup>1</sup> we dealt with experiments upon the effect of supplementing a basal diet with cold alcoholic extracts or dried corn (germinated and ungerminated) and found that such extracts contained little, if any, vitamine. We also fed undried varieties of corn (ungerminated, germinated and green). These acted, after a time, as if they contained a toxic element, often inducing loss of weight, eye trouble, paralysis and ultimate death. Our present experiments deal with the effect of supplementing a basal diet with *dried* samples of ungerminated, germinated and green corn. The object of these experiments was to see whether any growth-promoting substances, etc., were produced in the course of germination and greening.

The methods employed were essentially those already described (see previous paper). The experiments with the first ten groups of rats were started simultaneously and carried out under such identical conditions as to make the results, from the *comparative* standpoint, suggestive. During the first month each group of rats — four to a cage — receiving either ungerminated, germinated or green material, was given four grams of such material per day; during the second month this was increased to eight grams, and during the third month, to twelve grams. (We were forced to terminate these experiments at the end of the eleventh week.) The corn was carefully mixed with about one-half the quantity of total synthetic food consumed by the group per day and the mixture offered to the rats. Not until this mixture was entirely consumed was any more synthetic food offered.

Considering, first, the results at the end of the first month, it will be seen from the table (as well as from the curves) that, aside from the group on the "regular" diet (1), the one showing

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\* Paper presented before Soc. Exp. Biol. and Med., (21, 1924, 232).

the most notable increase in weight is group 10 [synthetic + *B* (yeast) + green]. We were anxious to confirm these results and we therefore ran a second series of experiments (11, 12, and 13, representing three groups of rats from one litter); here again it is evident that the group fed on synthetic + *B* + green showed the greatest increase in weight. Since neither 6 (synthetic + *B* + ungerminated) nor 8 (synthetic + *B* + germinated) show corresponding gains, the most obvious explanation is that a "growth" of vitamine (*A*?), or increase in the quantity present, has taken place during the course of "greening." This view would fall in line with suggestions made by several workers in the field (for example, Coward and Drummond<sup>2</sup> and Wilson)<sup>3</sup>. But another explanation offers itself as a result of the following experiment:

We carefully selected 100 seeds each of the ungerminated, germinated and seedling varieties and weighed them before and after air-drying, with the following results (weight in gm.):

	Fresh wt.	Dry weight (room temp.)	% dried material
Ungerminated	41.1	21.6	52.5
Germinated	46.2	24.1	52.1
Green (chlorophyllic) seedling	93.8	13.5	14.4*

In our experiments, following the methods of other workers, we took the *same* quantities of the different varieties of *dried* material and compared results; whereas, it would seem, the more logical procedure would have been to take quantities in the proportion of 21.6, 24.1 and 13.5. In other words, instead of taking 4 gm. ungerminated, 4 gm. germinated and 4 gm. green, we ought, perhaps, to take 4 gm. ungerminated,

$$\frac{4 \times 24.1}{21.6} \text{ gm. germinated (which is}$$

$$\text{about the same), and } \frac{4 \times 13.5}{21.6} \text{ gm. green}$$

If this view be taken, then the rapid increase in weight noticed in groups 10 and 13 during the first month need not neces-

\* Prof. R. A. Harper has kindly referred us to a paper by J. F. Breazale, in the *Jour. of Agric. Research*, 24, 1923, 41, in which somewhat similar results are recorded with wheat.



sarily be interpreted as showing growth of vitamine at all; and the positive results claimed by other workers must be questioned. These results are, of course, not to be interpreted as meaning that there is no growth of vitamine during germination and greening, but merely to suggest that the methods employed are open to criticism.

An examination of chart II will reveal another interesting fact: 5, 7, and 9, representing the weight curves of rats, the diet of which included one form of corn and *A* (cod liver oil) but no *B* (yeast), begin to gain markedly over 6, 8 and 10 (a diet including one form of corn and *B* but no *A*) from the sixth week on. It would seem, therefore, that, comparatively speaking, the seeds (ungerminated, germinated and green) are richer in vitamin *B* than in vitamin *A*; for in 5, 7 and 9, whatever *B* the rats get must come from the corn, just as in 6, 8 and 10, whatever *A* they get must come from the corn.

We are now engaged in comparing the effects of *equal weights* of dry material (ungerminated, germinated and green) with the effects produced by using the *same number* of seeds (ungerminated, germinated and green) and the effects produced by using the weights obtained from the ratio:

$$\begin{array}{ccc} 4 & : & 4 \times 24.1 : 4 \times 13.5 \\ \hline & & 21.6 \qquad 21.6 \end{array}$$

*Conclusions:* — It is plausible to assume that there is an increase in the amount of one or more vitamins in the course of "greening," and the work described in the paper, carried out along the lines described by other authors, points to an increase in vitamine *A*. It is shown, however, that the methods so far employed do not lead to conclusive results.

#### EXPLANATION OF TABLE.

Regular = Regular diet (on successive days, cabbage and cheese; meat and corn; bread and milk; cabbage and meat; turnips and cheese; bread and rye; meat and oats).

S = Synthetic diet (casein — extracted three times with hot alcohol and heated at 108° for 48 hours — 18 gm., corn starch; (Duryea's); 54 gm. lard — heated and oxidized for 24 hours — 24 gm.; salt mixture 4 gm.; water

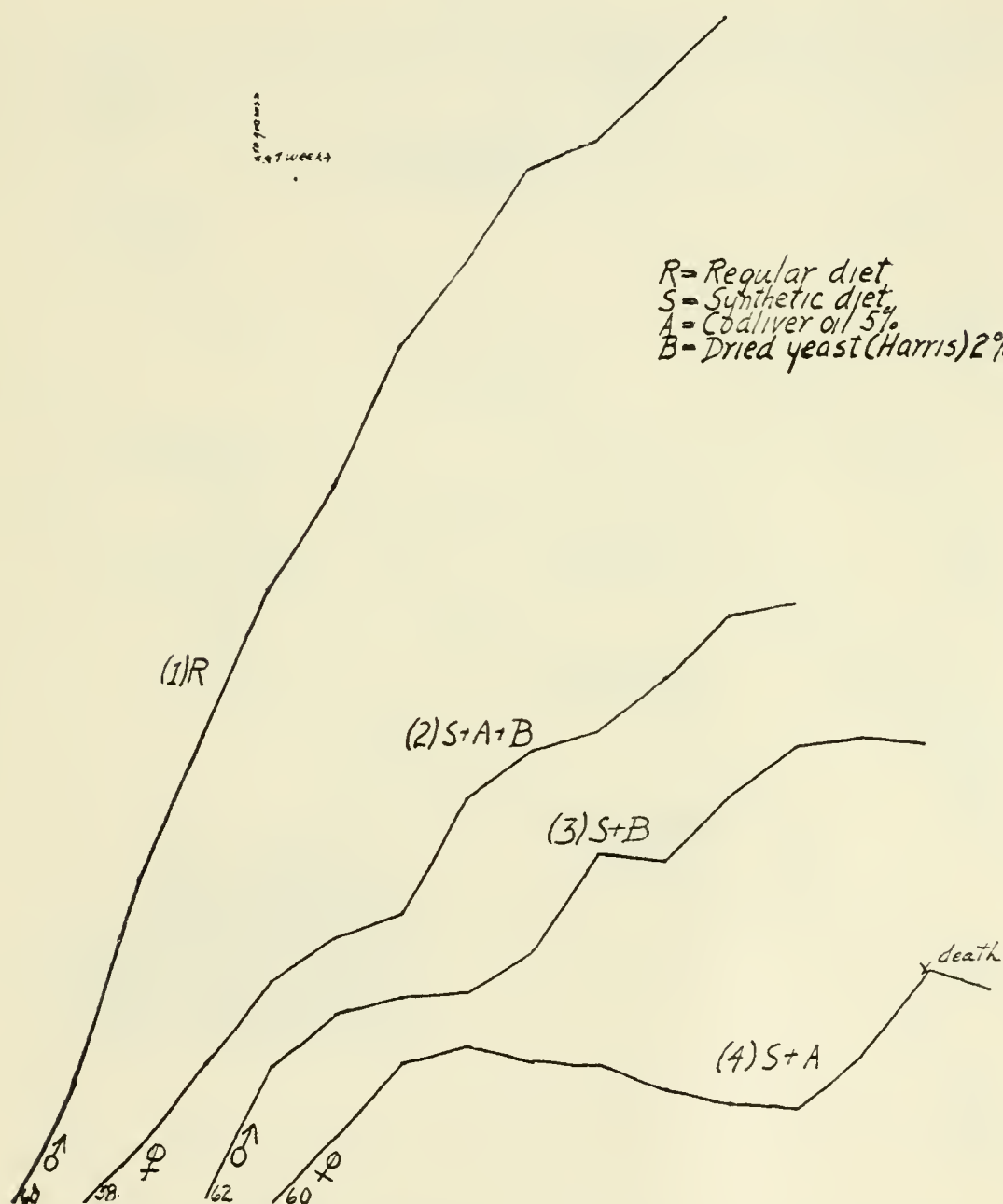


300 cc.) Fresh portions of synthetic diet were made up every other day, and when not in use, the food was kept in the ice-box.

*A* = Codliver oil, 5%

*B* = Dried yeast (Harris), 2%

*U* = Dried ungerminated corn



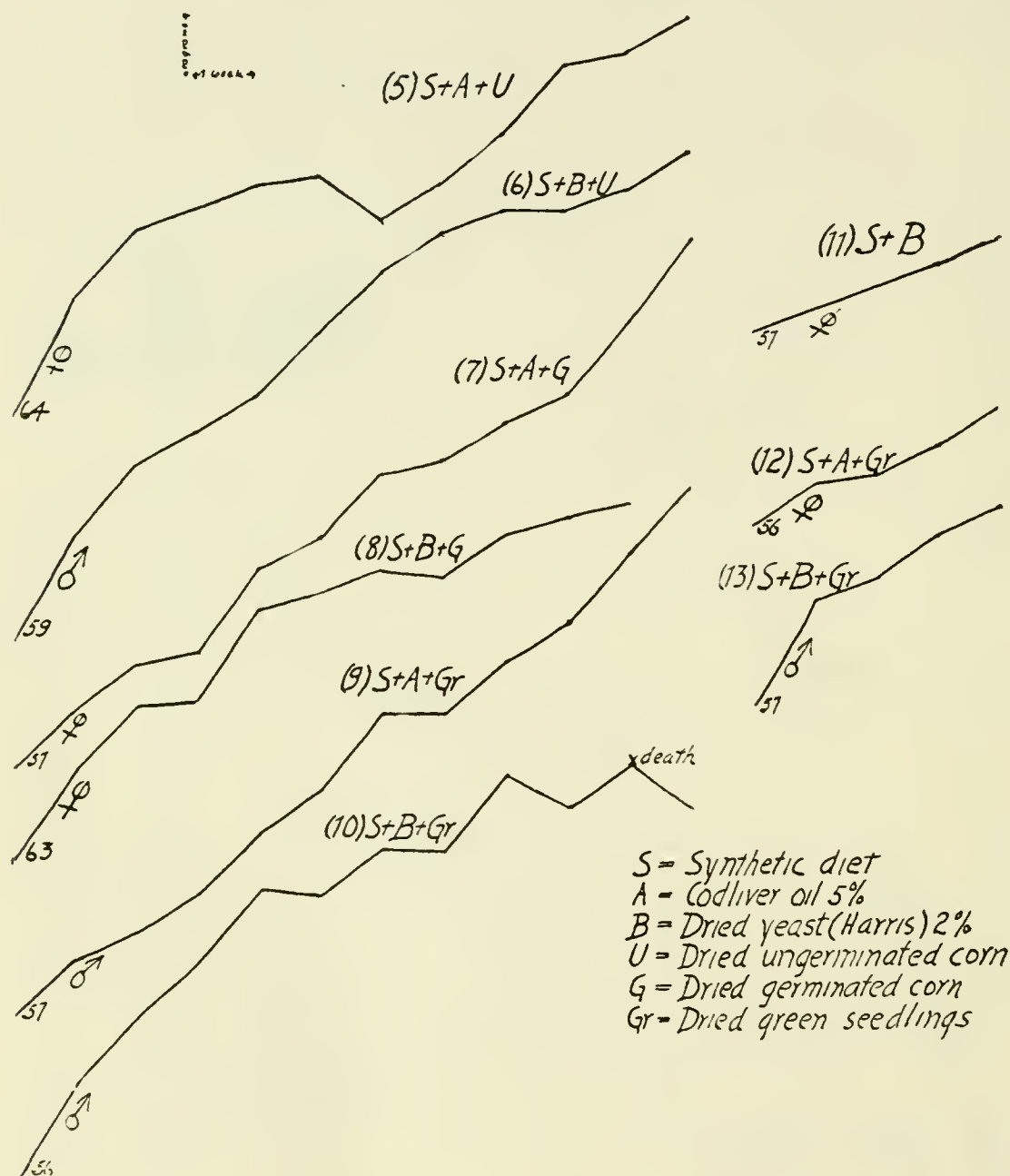
*G* = Dried germinated corn

*Gr* = Dried green seedlings

*Method of germination:*—The corn was kept in water overnight and then divided into three parts: part I was air-dried and ground, constituting



the "ungerminated corn." Part II was spread on moist filter paper, which in turn was placed over moist sphagnum that had been boiled 6 to 8 hours and thoroughly washed; the corn was next covered with moist filter paper and a thin layer of moist sphagnum. Sprouting occurred in three to four



days. The seeds were now air-dried and ground, representing "germinated corn." Part III was spread over thoroughly boiled and washed sphagnum (moist) in flower pots and covered with a thin layer of moist sphagnum. The pots were placed in the sunny part of the room and the sphagnum

sprayed with water from time to time. In favorable weather the advanced seedling stage, with two to three leaves, was reached in five to six days. The seeds and leaves were separated from the sphagnum, dried and ground. These constituted the "green seedling" stage.

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# THE SIGNIFICANCE OF VITAMINES IN ANIMAL NUTRITION

## EXAMINATION OF THE PRINCIPAL POULTRY AND DAIRY FEEDING-STUFFS FOR THEIR VITAMINE CONTENTS (A AND B)

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There is now general recognition of the fact that the vitamine content has a powerful influence upon the growth, fecundity, resisting power, and general health of domestic animals and upon the quantity and the food value of the products obtained from them, such as milk, butter, eggs, lard, etc. As this factor is naturally provided for in animals which have their freedom in choosing food (cf. Osborne and Mendel<sup>87</sup>) but may be seriously wrong in animals which are kept in confinement or on arbitrarily selected diets, some investigators have pointed out the necessity of making a wide examination of the vitamine contents of all the different feeding stuffs used for poultry, cattle and other domestic animals. Some references to this subject are contained in the appended bibliography, and others are given in standard text books.

In our investigation, a systematic analysis of each vitamine, A and B, was carried out on all the following feeds:

1. Yeast meal
2. Fish meal
3. Clover meal
4. Copra cake
5. Palm kernel cake
6. Linseed cake
7. Soybean cake
8. Poppy cake
9. Sesame cake

10. Rapeseed cake
11. Rangoon (ground-nut) cake
12. Semi-decorticated Mexican cotton-seed cake
13. Egyptian cotton-seed cake

Some of these meals have been previously analyzed by different investigators, and mention will be made of their results, under individual headings, in the following pages.

It will be noted that the majority of the meals come from vegetable sources, especially from oil-bearing seeds.

Though vegetable oils, as a rule, have been found to be deficient in vitamins, as the work of Drummond and Coward<sup>27</sup> and also of Drummond and Zilva<sup>32</sup> shows, nevertheless, when we considered the statement made by McCollum<sup>77</sup> we thought it worth while to test the sources from which the vegetable oils were obtained. McCollum in that paper states that vitamin A, while fat-soluble as obtained from animal sources, is not removed with the fat of the vegetable tissues. (cf. also Sherman<sup>102</sup>.)

Taking this fact into account, and also the fact that oil, in almost all of the above-mentioned cakes, has been removed by "expression," i. e., by pressure only and not by extraction, it may be assumed that these cakes should retain some vitamins if any were present.

The so-called "concentrates" also deserve to be examined. Osborne and Mendel<sup>88</sup> raised the point that most of the feeding stuffs do contain sufficient vitamins for the normal growth of rats, provided they could be made to eat large quantities of these feeds, and thus we come face to face with the problem of "vitamin-dilution" in nature. For our purpose, however, we shall only consider those "concentrates" as satisfactory which contain the necessary amount of vitamins for normal growth, in a comparatively small mass of the feed, not exceeding that sufficient for ordinary daily consumption.

## EXPERIMENTAL RESULTS

The technique adopted in analyzing for vitamins was the same as that used by us previously<sup>45</sup>. This method had originally been proposed by Drummond and Coward<sup>28</sup> and later described in detail by Drummond and Watson<sup>31</sup>.

In our researches in connection with vitamin A, only young rats, four weeks old and over 50 grams in body weight, were selected and put on a diet freed from vitamin A.

In about four weeks' time these rats became steady in their body-weights, and then each day one gram for every rat of the substance to be examined was added to the regular "A-deficient diet." This was generally done by kneading up the weighed quantity of the substance with a small piece of the basal diet, and this dough was given to the rat in the morning before it received the main bulk of its food.

When the amounts of these special stuffs had to be given in quantities larger than one gram a day, it was found more convenient to combine these with the basal diet of the rats, in order to ensure proper intake of these special substances.

It should be mentioned here that some of the young rats fed on "A-deficient diet" did not stop growing, and those which had continued to grow, and had attained more than 100 grams in body weight, were rejected by us for experimental purposes.

Generally four rats were put in one wire cage, and were watered by the inverted bottle method.

The "A-deficient diet" was made up as follows:

Purified Casein .....	20 parts
Purified Starch .....	50 "
Salt mixture .....	5 "
Hardened fat .....	10 "
Strained lemon juice .....	5 "
"Marmite" (autolysed yeast) .....	5 "

The casein which was used had been previously heated in thin layers exposed to air, in electrically heated ovens, at about 110 degrees Centigrade, for 24 hours, to destroy all vitamine A.

The hardened fat was obtained by the hydrogenation process from vegetable oils, and on account of the very high temperature used in its preparation, it was free from vitamins.

The rats which had been fed on "B-deficient diet" became steady in their body-weight much more quickly than those on "A-deficient diet." Only those rats whose body-weight was between 60 and 80 grams were put on "B-deficient diet," as it had been found that rats weighing less died off very quickly on "B-deficient diet."

The composition of the "B-deficient diet" was as follows:

Extracted casein .....	20 parts
Extracted starch .....	50 "
Salt mixture.....	5 "
Butter .....	10 "
Strained lemon juice .....	5 "

Casein, used in the preparation of the "B-deficient diet," had been previously extracted several times with alcohol and ether. This process, first used by Funk and MacCallum<sup>44</sup>, removes all the vitamins.

The salt mixture used by us was a modified "McCollum salt mixture." Its composition was as given below:

Sodium chloride .....	5.19 grams
Sodium acid phosphate .....	10.41 "
Magnesium sulphate .....	7.98 "
Potassium phosphate .....	28.62 "
Calcium lactate .....	39.00 "
Calcium phosphate .....	16.02 "
Ferric citrate .....	3.54 "



Our results are summarized in the accompanying table:

EXPERIMENTS ON		(1) "A - DEFICIENT DIET"			(2) "B - DEFICIENT DIET"		
Name of Meal		Dose Grams.	Growth	Value as a source of Vitamin A.	Dose Grams.	Growth	Value as a source of Vitamin B.
1.	Yeast meal.....	1-2	Slow	Small amount	1	Very good	Very good
2.	Fish meal.....	1.5	Slow	Present	1-2	Slow	Small
3.	Clover meal.....	1-2.5	None	Nil	2.5-3	Fair	Fair
4.	Copra meal.....	3	Slow	Small amount	2	Growth	Fair
5.	Palmkernel cake...	2	None	Nil	3	None	Nil
6.	Linseed cake.....	2	Fair	Good	2	Fair	Fair
7.	Soybean cake.....	2	Growth	Present	2	Good	Good
8.	Poppy cake .....	1.5	Fair	Fair	1.5-2.5	Fair	Fair
9.	Sesame cake .....	2.5	Very small	Very little	3	None	Nil
10.	Rapeseed cake....	2	Small	Small amount	2	Growth	Present
11.	Rangoon cake .....	2.5	Fair	Fair	3	None	Nil
12.	Mexican cake .....	2	Fair	Fair	2	Growth	Fair
13.	Egyptian cake ....	2.5	Nil	Nil	2	Growth	Present

(1) EXPERIMENTS WITH YEAST MEAL

Source: As a commercial by-product in the brewing industry, the yeast is pressed into cakes, and dried at a temperature sufficiently high to destroy all the enzyme. The yeast meal is a dry powdery substance of a brownish color.

Some investigators have determined the nutritive value of dry yeast.

(cf. Bull. 73, University of Leeds and York, Council Agr. Educ. 1922.) Hancamp<sup>59</sup> states that in his fattening experiments with sheep and hogs, he found yeast meal to be very easily digested by animals, and especially suited for raising and fattening swine.

Here mention may be made of Abderhalden and Schaumann's work<sup>2</sup>. They found 0.5 grams of yeast per day sufficient for the maintenance of body weight in rats.

The composition of the sample examined by us was:

Water .....	4.25 %
Protein (Nx6.25) ....	41.88 %
Fat .....	5.09 %
Ash .....	7.05 %
Other ingredients ....	40.47 %

It may be mentioned that this sample of yeast meal was one of those obtained as a commercial by-product and fermentation experiments showed the yeast to be all dead.

The results we obtained showed that dried yeast is deficient in vitamine A. Osborns and Mendel<sup>92</sup> also failed to find any appreciable amount of vitamine A in it; but this yeast meal is a remarkably good source for vitamine B.

## (2) EXPERIMENTS WITH FISH MEAL

*Source:* Fish remains are dried, and then ground up and sold as "fish meal." This is sometimes made from good white fish when there is a glut in the market, but more often very inferior material is used for this purpose.

The composition of the different fish meals examined by us varied, though not widely, and the table below shows the composition of the samples examined:

	Moisture	Protein	Fat	Ash
Sample 1.....	12.2%	48.5 %	7.7 %	20.7%
Sample 2.....	12.5%	54.38%	4.05%	23.0%

The amount of digestible substance in fish meal has been examined by some investigators, and it has been recommended that fish meal should preferably be used with some carbohydrate feed. (Crowther<sup>18</sup> and Bull. 73, Univ. of Leeds and York, Council of Agr. Edc. 1922.)

That fish itself may contain some vitamine B has been shown by Drummond<sup>24</sup>. Different fish-liver oils have been shown to be a very potent source of vitamine A. Therefore it is supposed that fish meal may contain some vitamine A as well as vitamine B.

Our results indicate that fish meal furnishes a fair quantity of vitamine A but very little vitamine B.

## (3) EXPERIMENTS WITH CLOVER MEAL

*Source:* Clover is generally grown as a rotation crop, and the chief purpose of this "rotation" is to enrich the nitrogen content of the soil. Clover meal is the air-dried, chopped up pieces of the plant.

The composition of the sample was as given below:

Moisture	Protein	Fat	Ash
9.0%	13.1%	2.0%	2.1%

Osborne and Mendel<sup>91</sup> found both vitamins A and B in dried clover. Steenbock and Gross<sup>103</sup> obtained similar results. They showed that 5-10% of clover in the diet provided sufficient vitamin A for normal growth in rats; the amount of vitamin B was, however, less, and 15-20% of clover in their diet was necessary to make rats grow when deprived of vitamin B.

The results arrived at by us were also the same.

## (4) EXPERIMENTS WITH COPRA CAKE

*Source:* Copra cake is obtained as a commercial by-product in the cocoanut oil industry. After the oil has been removed by pressure, the remains of the copra are sold as "copra cake."

The composition of the sample examined was:

Moisture	Protein	Fat	Ash
11.0%	21.1%	9.1%	5.8%

John, Finks and Paul<sup>62</sup> have studied the nutritive value of cocoanut globulin and cocoanut press-cake. They found both vitamins A and B in copra cake. Alvarez<sup>6</sup>, however, stated that his experiments showed copra meal itself as not producing much growth, but when supplemented with green leaves this meal proved to be an excellent feeding stuff. On account of the presence of different amino-acids such as lysine, etc., Jansen<sup>66</sup> had also examined the cocoanut press-cake for its nutritive value.

Honcamp<sup>60</sup> observed that, though copra meal did not increase the total amount of milk in cows, yet when this was eaten in large quantities the percentage of milk-fat increased favorably.

Superiority of the proteins of the cocoanut-oil meal was also demonstrated by Maynard and Fronda<sup>74</sup>. Likewise McClandish and Weaver<sup>78</sup> found that, despite its lower content of total protein, copra meal has a higher feeding value than many other meals, e. g., linseed oil meal, peanut meal, gluten meal, and soybean meal.

Our results indicate that copra meal furnishes a small quantity of vitamin A but a fair amount of vitamin B.



## (5) EXPERIMENTS WITH PALM KERNEL

*Source:* From African palm nuts after the oil has been removed.  
The composition of the sample examined by us was:

Moisture	Protein	Fat	Ash
11%	18.6%	7.8%	3.9%

Palm oil has been shown by Drummond and Coward<sup>15</sup> to contain fairly large amounts of vitamine A. The nutritive value and the composition of palm kernel cake have previously been examined by other investigators. (Cf. Crowther<sup>19</sup>.) It has been found to contain some vitamine A by Stammers<sup>106</sup>. Since palm kernel cake is a by-product in the palm oil industry, it was thought that the cake might also contain some vitamine A. Our results, however, show that this cake is very deficient in vitamins.

In spite of the absence of appreciable quantities of vitamins in palm kernel cake, it has been considered by Crowther and Woodman<sup>20</sup> to be superior to cotton-seed meal as a feeding stuff.

## (6) EXPERIMENTS WITH LINSEED OIL

*Source:* Linseed oil is largely used in the paint industry. After the oil has been removed, the pressed remains are sold as "linseed cake."

The composition of the sample examined by us was:

Moisture	Protein	Fat	Ash
10.8%	31.8%	9.3%	5.8%

Linseed cake has long been valued as an extremely satisfactory food in the dairy industry. It has been stated by Kellner<sup>99</sup> and by Armsby<sup>8</sup> that the administration of linseed cake improves the milk yield. Drummond and Coward<sup>21</sup> have shown that linseed oil may contain appreciable amounts of vitamine A.

Our results indicated substantial quantities of both vitamine A and vitamine B in the linseed cake.

## (7) EXPERIMENTS WITH SOY BEAN CAKE

*Source:* As a by-product in the soy oil industry; also specially grown in Russia, China and Japan to furnish a feed for cattle. Soy bean cake is sold in the market after the oil has been removed by expression, as well as by extraction.

The composition of the sample examined was:

Moisture	Protein	Fat	Ash
14.5%	42.4%	7.0%	5.3%

Osborn and Mendel<sup>87</sup> reported the presence of a considerable amount of vitamine B in soy bean cake. According to them, it is questionable if any vitamine A is present in these cakes. Daniels and Nichols<sup>22</sup>, however, found appreciable quantities of vitamine A in soy bean cakes. Abderhalden and Schaumann's<sup>4</sup> work on pigeons confirms these previous find-

ings. Eddy<sup>36</sup> also mentions the presence of vitamins A and B in soy beans.

The nutritive value of the proteins in soy bean cake has been found by Abderhalden<sup>1</sup> fairly good. Johns and Finks<sup>38</sup> studied later the nutritive value of soy beans and confirmed the previous findings.

The results obtained by us indicate the presence of vitamins A and B in soy bean cakes.

#### (8) EXPERIMENTS WITH POPPY CAKE

*Source:* This is obtained as a by-product in the opium industry and poppy-seed oil production. The oil is removed by crushing the seeds and then applying pressure.

The composition of the sample examined was:

Moisture	Protein	Fat	Ash
8.5%	41.2%	7.1%	5.3%

Some workers have regarded poppy cake as unsuited for cattle feed generally. Kellnar<sup>39</sup> says that animals fed on poppy cake diet manifest a tendency to remarkable slowness and sleepiness. This soporific effect of the diet, according to him, is due to small quantities of opium contained in it. The work of Annett and Sen<sup>7</sup> is of interest in this connection. By experimenting on cattle they found that poppy seed diet did not act adversely on the composition of milk or butter-fat. Moreover, the cake was eaten readily, and no ill effects were noticed.

In our experiments with rats, no ill effects were apparent, and the results obtained indicate the presence of both vitamins A and B.

#### (9) EXPERIMENTS WITH SESAME CAKE

*Source:* Sesame cake is a by-product in the sesame oil industry. The oil is extracted from the seeds by subjecting them to one cold pressure, followed by two warm pressures. The cake is used as cattle feed.

The composition of the sample examined was:

Moisture	Protein	Fat	Ash
9.4%	42.1%	12.2%	9.8%

It appears that the examination of sesame cake itself for the presence of different vitamins has not been done before. Its nutritive value has been investigated before, in which connection reference may be made to Crowther's work<sup>10</sup>.

The results obtained by us indicate an absence of appreciable quantities of vitamins A and B in the sesame cake.

## (10) EXPERIMENTS WITH RAPE SEED CAKE

*Source:* This is obtained as a commercial by-product in the rape oil industry. The oil is removed from the cakes by pressure or by extraction.

The composition of the sample examined was:

Moisture	Protein	Fat	Ash
10.1%	33.1%	10.2%	7.7%

Van Kampen<sup>11</sup> has reported that sometimes an exclusive feeding of rape seed cake causes sickness in cattle. He does not, however, mention whether this sickness is a form of a deficiency disease or not.

The results obtained by us show that rape seed cake may contain some vitamine A as well as B, though these amounts are not large.

## (11) EXPERIMENTS WITH RANGOON (Ground Nut) CAKE

*Source:* Rangoon beans are especially grown for the purpose of cattle feed.

The composition of the sample examined was:

Moisture	Protein	Fat	Ash
9.6%	48.4%	7.2%	5.2%

Some investigators have found that the Rangoon nut has acted like poison when farm animals have been fed on it, and Berg<sup>9</sup> is one of these. It has later been shown that during the preparation of the cake the toxic matter is completely destroyed, and in this connection we may cite the work of Whittle and Rheinberger<sup>14</sup>. Drummond and Zilva<sup>12</sup> found no appreciable quantity of vitamine A in the rangoon cake.

The results obtained by us also indicate an absence of vitamine A as well as vitamine B.

## (12) EXPERIMENTS WITH SEMI-DECORTICATED MEXICAN COTTON-SEED CAKE

*Sources:* Both kinds examined are obtained as by-products in the cotton seed industry. After the cotton has been removed, the seeds are pressed to yield their oil contents. The pressed lumps are sold as "cotton seed cake."

The compositions of the samples examined were:

	Moisture	Protein	Fat	Ash
(a)	8.0%	33.1%	5%	7.7%
(b)	12.0%	23.0%	5.5%	5.9%

Cotton seed cakes contain, as a rule, a toxic substance, which when consumed by pigs brings about their death in fifty to sixty days. Roberts<sup>18</sup> as well as Rommel and Vedder<sup>19</sup> claim that a sort of deficiency disease is produced in pigs when fed on cotton seed meal.

Drummond and Zilva<sup>12</sup> found that the toxic substance in cotton seed meal brought about death in a few days in experimental rats.



Carruth<sup>10</sup> has worked on the toxicity of different cotton seed products. According to him, the relative toxicity of cotton seed meals varied with the extent to which the raw cotton seed had been cooked in the oil mill. He found that cotton seed is highly toxic, and contains about 0.6 per cent of gossypol. During the hot pressing and preparation of the cotton seed meal, the glands containing gossypol are disintegrated, and gossypol becomes spread over the surface of the seed tissue, thus apparently undergoing oxidation and becoming harmless. This oxidized gossypol is no longer oil- and ether-soluble.

Carruth suggests that the sulphuric acid test for unchanged gossypol should be made before cotton seed meals are given to animals. The test consists in the development of red areas where unchanged gossypol of the cotton seed comes in contact with concentrated sulphuric acid.

The works of those investigators who found "prepared" cotton seed meal not injurious, but of really good nutritive value, may be mentioned here. Nevene<sup>85</sup> found no toxic symptoms in rats fed on cotton seed meal. Richardson and Green<sup>97</sup> state that cotton seed flour, when amounting to 50 % of the diet, contains sufficient water-soluble vitamines for normal growth. This, however, does not contain sufficient vitamine A, but when an extract from cotton seed meal is given, normal growth is obtained. Ahrens<sup>5</sup> found that cotton seed meal could be favorably compared with beef scraps. Daniels and Loughlin<sup>21</sup> report that cotton seed meal may contain good quantities of vitamines. Reference may also be made to the work of Coombs and Curtis<sup>14</sup>, who examined the effect of cotton seed meal on the growth and production of cows.

The results obtained by us indicate the presence of no toxic substance in the two samples of cotton seed meal, and both vitamines A and B were detected, though only in small quantities.

#### DISCUSSION AND GENERAL SUMMARY

An attempt has been made in this work to determine the vitamine contents of some of the principal feeding stuffs used for ordinary farm purposes. The object has been to find out which of the feeding stuffs are especially rich in vitamines, in order that, by the proper administration of food rich in vitamines, the malnutrition and deficiency diseases in domestic animals may be prevented.

Such diseases, as is now well known, do not occur when the animals are living under natural conditions and are obtaining ample supplies of green fodder; but under other circumstances it appears very important to pay particular attention to the vitamine A intake of domestic animals, largely on account of the fact that many of the ordinary farm "concentrates" tend to be somewhat deficient in this vitamine.

We have found fish meal, poppy cake, linseed cake, and clover meal to be specially rich in vitamine A, and, with the exception of the fish meal, all of these contain important quantities of vitamine B as well. Proper addition of one or more of these meals to an ordinary diet of dry fodder will doubtless have very beneficial effects.

The high biological food value of linseed cake is of interest. For generations farmers and cattle breeders have realized its value, and this meal is often given to improve the general condition of the animals. Kellner<sup>69</sup> has emphasized its high value as food for diseased cattle. We have already mentioned Drummond and Zilva's<sup>32</sup> observations on the relatively high amount of vitamine A in linseed cake, which agree with McCollum's<sup>78</sup> finding. The results obtained by us also bear this out.

Clover meal also contains very good quantities of the different vitamins, and this meal should be widely used.

Poppy cake should also be utilized, since, owing to its moderate content of both vitamins A and B, it is an extremely desirable feed.

Yeast meal, though an extraordinarily good source for vitamine B, is deficient in vitamine A, but the great advantage of this meal as a feeding stuff lies in the fact that the protein contained in it is almost 100 per cent digestible, and is of high biological value. It should be of great assistance to farmers, not only in the fattening of their stock, but also for warding off the different forms of bovine beri-beri which occasionally break out.

Rape seed cake may be considered a fairly good feed. It is not very rich in vitamins, but yet contains appreciable amounts of both A and B.

Soy bean cake too is to be recommended as a good source of vitamine B. Its protein is regarded as of high biological value.

The two cotton seed cakes, i. e., semi-decorticated Mexican cotton seed cake and Egyptian cotton seed cake, are found to have fair amounts of vitamine B, although the amount of vitamine A is relatively low. Toxic effects were not observed in our rats, and can probably be avoided in farm stock if the

precaution is taken of testing with sulphuric acid for the presence of gossypol.

Copra cake contains some vitamine B. Alvarez<sup>6</sup> and Honcamp<sup>60</sup> consider that this meal, though not growth-promoting, exerts a beneficial influence toward increasing the fat-content of the milk, and also in other directions, since copra cake has been proved to contain some of the more important amino acids, like lysine, etc. As Hart and Humphrey<sup>51</sup> have found that, for meeting the protein requirements of milch cows, the protein of the "oil-meals" is 50% more efficient than the proteins of the corn (maize) or of the wheat kernel, for this reason also copra meal should be recognized as a specially suitable feed.

Palm kernel cake has scarcely any vitamine A or B in it.

Sesame cake and Rangoon (ground-nut) cake are apparently quite devoid of any vitamins detectable by the available biological method. We agree with the majority of investigators that Rangoon (ground-nut) cake has a very low nutritive value, and hence we regard it as rather undesirable feeding stuff.

Sesame cake has no injurious effect, nevertheless its low nutritive value makes it an undesirable feed.

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# THE EFFECT OF GERMANIUM DIOXIDE IN EXPERIMENTAL ANEMIA WITH OBSERVATIONS ON THE ACTION OF THIS COMPOUND IN PRODUCING KIDNEY INJURY.

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In a study of the physiological action of germanium dioxide, Hammett, Nowrey and Müller<sup>1</sup> observed that the subcutaneous injection of this compound into mature albino rats produced a marked and sustained rise in the number of erythrocytes. The resultant erythrocythemia is said to have been approximately the same whether small (total of 6.6 mg. per kilo. of body weight) or large doses (total of 45 mg. per kilo.) were administered. Subsequently Hammett and Nowrey<sup>2</sup> presented additional data in support of their view that germanium dioxide is a potent erythropoetic agent.

The use of germanium dioxide in the treatment of anemia has been suggested as a result of this work as well as that of a number of other investigators, namely, Kast, Croll and Schmitz<sup>3</sup>, Schmitz<sup>4</sup>, Lenker<sup>5</sup>, and Müller and Iszard<sup>6</sup>. These have reported beneficial results in anemia. In the thirteen unselected cases of anemia in childhood studied by Schmitz<sup>4</sup>, increase in the number of erythrocytes were observed in all but a case of von Jaksch's anemia. On the other hand, Alexander<sup>7</sup> observed no clinical improvement or increase of hemoglobin or erythrocytes in three cases of pernicious anemia. The subject of germanium dioxide therapy has been judiciously reviewed by Minot and Sampson<sup>8</sup> who, as a result of a critical analysis of the work of others as well as of their own observations, conclude that no evidence has been obtained suggesting that germanium dioxide has the power to increase blood formation or that it exerts any significant effect to improve the health of anemic individuals and that consequently the drug seems to be of no value in the treatment of anemia.

There appears to be general agreement that germanium dioxide is non-toxic. A comparison of the relative toxicity of germanium dioxide and arsenic trioxide has been undertaken by Hammett, Müller and Nowrey<sup>9</sup>

who found that the former compound could be administered in doses up to 180 mg. per kilo. of body weight to albino rats without producing harmful effects. Arsenic trioxide, given in much smaller doses (8 mg. per kilo.)

TABLE 1.

*Effect of Germanium Dioxide in Experimental Anemia.  
Dog 37, Male.*

Date 1923	Weight kilos.	Red count in millions	Hemoglobin %	Remarks		
3-25	4.40	5.28	81	0.2 cm.	Methylphenylhydrazine	
3-26		4.60	71			
3-27		4.60	70			
3-28		3.28	61	0.2 cc.		
3-29	4.20	3.52	60			
3-30		3.10	56			
4- 8	4.10	3.20	58			
4-10		3.20	58			
4-11		3.40	56	40 mg.	GeO <sub>2</sub>	subcutaneously
4-12		3.80	60	40 mg.	"	"
4-13		3.92	60			
4-14		4.30	64			
4-15		4.00	66	32 mg.	"	"
4-17		3.50	60			
4-18		3.60	65	40 mg.	"	"
4-19	4.00	3.30	63			
4-21		4.10	65			
4-23		4.00	63	65 mg.	"	"
4-24		3.80	59			
4-25		3.40	60			
4-27		3.36	60	120 mg.	"	"
4-28		4.40	62			
4-30		4.00	62	65 mg.	"	"
5- 2		3.84	60			
5- 5		4.16	62	80 mg.	"	"
5- 7		4.00	66	140 mg.	"	"
5- 9		4.28	68			
5-11		3.30	51			
5-16	3.68	3.20	50			



TABLE 2.

*Effect of Germanium Dioxide in Experimental Anemia.  
Dog 48, Male.*

Date 1923	Weight kilos.	Red count in millions	Hemoglobin %		Remarks	
4-13	7.50	5.05	86	75 mg.	sym. di-isopropyl hydra- zine hydrochloride	
4-16		4.48	77	160 mg.	"	
4-17		4.70	82	75 mg.	"	
4-21		3.60	54			
4-23	7.20	2.00	36			
4-24		1.90	29	80 mg.	GeO <sub>2</sub>	subcutaneously
4-25		1.84	30	80 mg.	"	"
4-29		2.50	45			
4-30	6.70	2.88	49	100 mg.	"	"
5- 2		3.48	56			
5- 5		4.16	63	80 mg.	"	"
5- 7		4.16	63	120 mg.	"	"
5- 9		3.60	58			
5-11		3.52	60			
5-17		3.68	62			
5-22		3.80	62			
5-23		3.80	60	120 mg.	"	"
5-24		4.24	61	120 mg.	"	"
5-25		4.16	62	120 mg.	"	"
5-27		3.68	60	120 mg.	"	"
5-29	7.30	3.76	60	120 mg.	"	"
5-31		3.84	60			
6- 2		3.64	58			

resulted fatally. Müller and Iszard<sup>6</sup> state that germanium dioxide does not accumulate in the tissues but that it is eliminated by way of the kidneys and alimentary tract. Lenker<sup>5</sup> likewise observed no toxic manifestations as a result of germanium dioxide therapy.

It does not appear that any serious attempt has been made by these workers to determine whether germanium might not resemble arsenic in its effect on the liver and kidney. We shall present evidence to show that germanium dioxide is injurious to the kidney glomeruli. The present work was undertaken with

TABLE 3.

*Effect of Germanium Dioxide in Experimental Anemia.  
Dog 44, Female.*

Date 1923	Weight kilos.	Red count in millions	Hemoglobin %	Remarks	
4-13	8.00	7.20	120	80 mg.	sym. di-isopropyl hydra- zine hydrochloride
4-15	7.60	7.20	108	80 mg.	"
4-18		6.40	100	160 mg.	"
4-21	6.60	5.20	85		
4-23		5.12	84	240 mg.	"
4-25		4.00	56		
4-28		2.88	46		
5-11		3.52	57		
5-23	4.40	2.80	43	120 mg. GeO <sub>2</sub>	subcutaneously per oz.
5-24		3.04	40	100 mg.	"
5-25		2.72	38	100 mg.	"
5-27		3.26	41	100 mg.	"
5-28		2.72	40	100 mg.	"
5-29		2.62	40	120 mg.	"
5-31		2.60	38	180 mg.	"
6- 2		2.72	38		

the object of studying the effect of germanium dioxide under somewhat more adequately controlled experimental conditions than is possible in clinical studies.\*

In a preliminary communication<sup>10</sup>, one of us reported the results obtained with germanium dioxide in the case of a puppy made anemic with methylphenylhydrazine. Complete results of this experiment are presented in Table 1. These data show that the administration of germanium dioxide over a period of one month did not produce any demonstrable improvement in the condition of this animal. The transitory increases observed at times are in no way associated with the action of germanium, for the administration of this compound was followed by a de-

\* The germanium dioxide used in this work was obtained from Professor L. M. Dennis of Cornell University to whom we are greatly indebted. This preparation was of very great purity and contained less than 1 part of arsenic in 200,000 parts of the dioxide, as determined by spectrophotometric measurements performed by Dr. J. Papish of Cornell University.

crease in the number of red cells about as frequently as by an increase.

The results of two additional typical experiments are presented in Tables 2 and 3. Severe anemia was produced in Dog 48 by means of symmetrical di-isopropylhydrazine hydrochloride<sup>11</sup>. When the red count had fallen to 1,800,000 germanium treatment was begun. For a period of a week, rapid red cell regeneration was noted. Subsequent injections did not affect the number of red corpuscles markedly. In interpreting these results, account should be taken of the animal's condition. At almost all times this dog appeared greedy for food, which was supplied in adequate amounts. In control experiments, well nourished animals have been found to recover with similar rapidity. We are therefore inclined to the view that the initial improvement was not due to the germanium.

In contrast to the behavior of Dog 48, Dog 44 refused food during the first week of the experiment. As a result, the animal lost considerably in weight. Continued oral administration of germanium dioxide did not affect the condition of this animal. In a general way, it may be said that the germanium treated anemic dogs showed no greater rate of red cell regeneration than untreated anemic dogs.

An attempt has been made to determine the effect of germanium dioxide on red cell regeneration in rabbits made anemic with phenylhydrazine. In chart 1 are represented the results obtained in the case of six anemic and three normal rabbits receiving periodic injections of germanium dioxide, and of six untreated anemic rabbits. It appears from our findings that germanium dioxide had no effect on red cell regeneration in the anemic rabbits. Likewise, no significant alterations in the red count were observed in the case of the normal rabbits receiving germanium dioxide.

#### RENAL INVOLVEMENT DUE TO GERMANIUM

The microscopic examination of the kidney tissue of one of the anemic dogs (Dog 37), which had received germanium dioxide, indicated that this compound may have exerted a destructive effect on the glomeruli. This animal had received a total of 622 mg. of germanium dioxide over a period of about



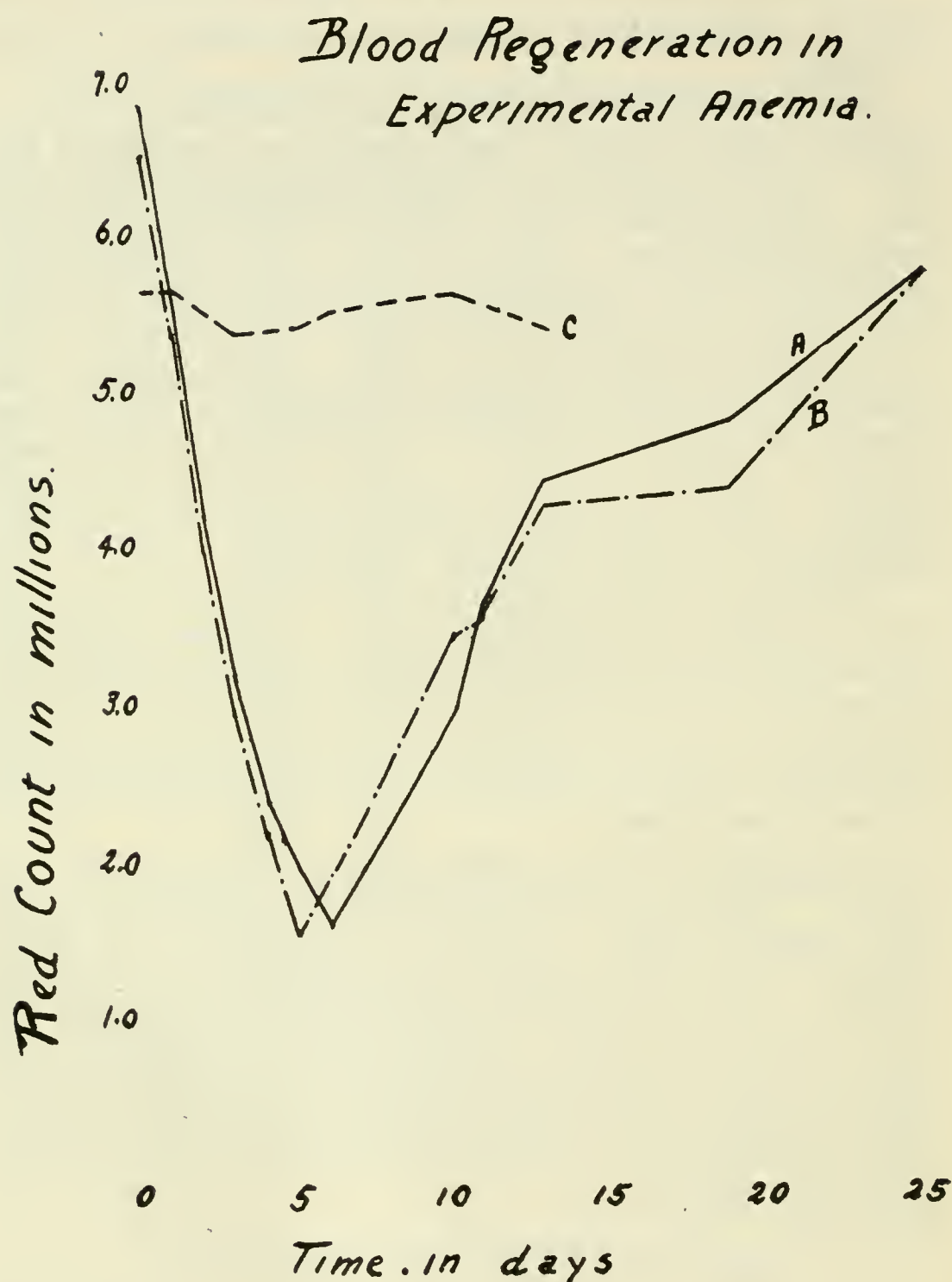


Chart legend:

Curve A represents the results obtained in the case of six anemic rabbits, each weighing 2.6 to 2.9 kilos. In each case, anemia was produced by the injection of 35 mg. of phenylhydrazine. A total of 280 mg. of germanium dioxide was administered to each rabbit in seven 40 mg. doses at intervals from the fifth to the twenty-fifth day.

Curve B represents the course of phenylhydrazine anemia in six rabbits, each weighing 2.6 to 2.9 kilos. Anemia was produced as in the first six rabbits but no germanium dioxide was administered.

Curve C represents the effect of germanium dioxide in three normal rabbits. Doses of 40, 80, 80, 130 and 120 mg. of germanium dioxide were administered to each rabbit on the 2d, 4th, 6th, 7th and 10th day of the experiment respectively.

one month. It will be observed from Figure 1 that all the glomeruli have a shrunken appearance, and that in the case of at least three or four of the glomeruli in the field destructive changes are definitely indicated. The possibility of post-mortem changes being responsible for these findings is excluded, as the kidneys were removed from the dog under slight ether anesthesia and suitably preserved.

These observations were confirmed in a normal dog which had received a total of 1.3 gm. of germanium dioxide over a period of about three weeks. As this animal served likewise as a control in determining the effect of germanium dioxide on the blood in normal animals, the data are presented in the following protocol:

Dog No. 61, male, weight 6.4 kilos. March 14; red count 6,560,000, white count 6,400; differential count, polymorphonuclears 84, lymphocytes 10, large mononuclears 0, transitionals 3, eosinophiles 2, basophiles 1, no nucleated red cells. Dog received subcutaneous injections of germanium dioxide, each containing 100 mg., on March 17, 18, 20, 25, 25, 26, 28. March 29: red count 6,120,000, white count 11,200; differential, polymorphonuclears 86, lymphocytes 4, large mononuclears 0, transitionals 8, eosinophiles 2, no nucleated red cells.

Dog received injections, as before, on March 29, 31, April 1, 3, 5. April 8: red count, 5,620,000, white count 11,000; differential, polymorphonuclears 77, lymphocytes 5, large mononuclears 5, transitionals 11, eosinophiles 2, no nucleated red cells. There was sloughing of the skin at the site of the injections. Dog received 200 mg. of germanium dioxide by stomach tube.

April 9: red count 5,700,000, white count 11,000, no nucleated red cells.

Dog killed and kidneys removed on April 9. Grossly, the bone marrow of this animal appeared somewhat redder than is usually observed in animals of approximately the same age. On microscopic examination the spleen was found to be normal in appearance. The liver showed a moderate granular change with early fatty change in the hepatic cells. Mild hyperplasia was observed in the bone marrow. Destructive changes of the tissues of the glomerular tufts were found in the kidney. There

was practically no tubular involvement. The contrast in appearance between a number of the injured glomeruli and some which had remained practically unaffected is brought out in Figure 2.

For the purpose of comparing the action of germanium with that of arsenic, arsenic poisoning was produced in two dogs. In both cases there was marked hyperemia in the glomeruli with hemorrhagic areas. There was also very marked involvement of the tubules. These observations are in accord with those of a number of other investigators. Karsner and Denis<sup>12</sup> have pointed out that early arsenic nephritis is associated in the cat with but slight glomerular injury. More severe involvement occurs in the tubular epithelium. Pearce, Hill and Eisenbrey<sup>13</sup> have shown that in the dog, arsenic nephritis is primarily of the vascular type during the early stages but that subsequently (within three to five days) it resembles more closely the tubular forms of nephritis. Our results with germanium dioxide indicate that this compound might be useful for the production of experimental glomerular nephritis.

### SUMMARY

1. Germanium dioxide does not appear to exert any beneficial effect in experimental anemia. Likewise, no significant changes in the blood have been observed in normal dogs and rabbits receiving germanium dioxide.

2. Evidence has been obtained to show that germanium dioxide is injurious to the kidney, the destructive effect being limited to the glomeruli. At least this is true for the doses used in our experiments.

In conclusion, the writers wish to express their indebtedness to Dr. W. H. Hill of the pathology department for the preparation of a number of photomicrographs in connection with this work.

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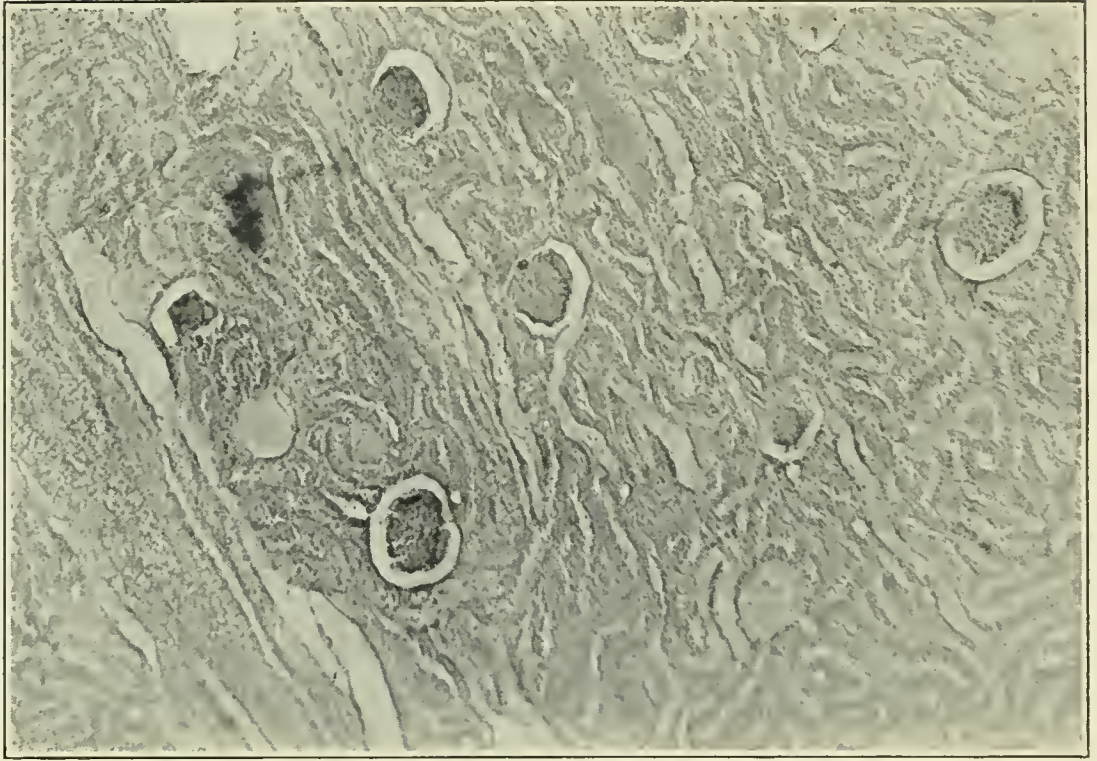


FIG. 1

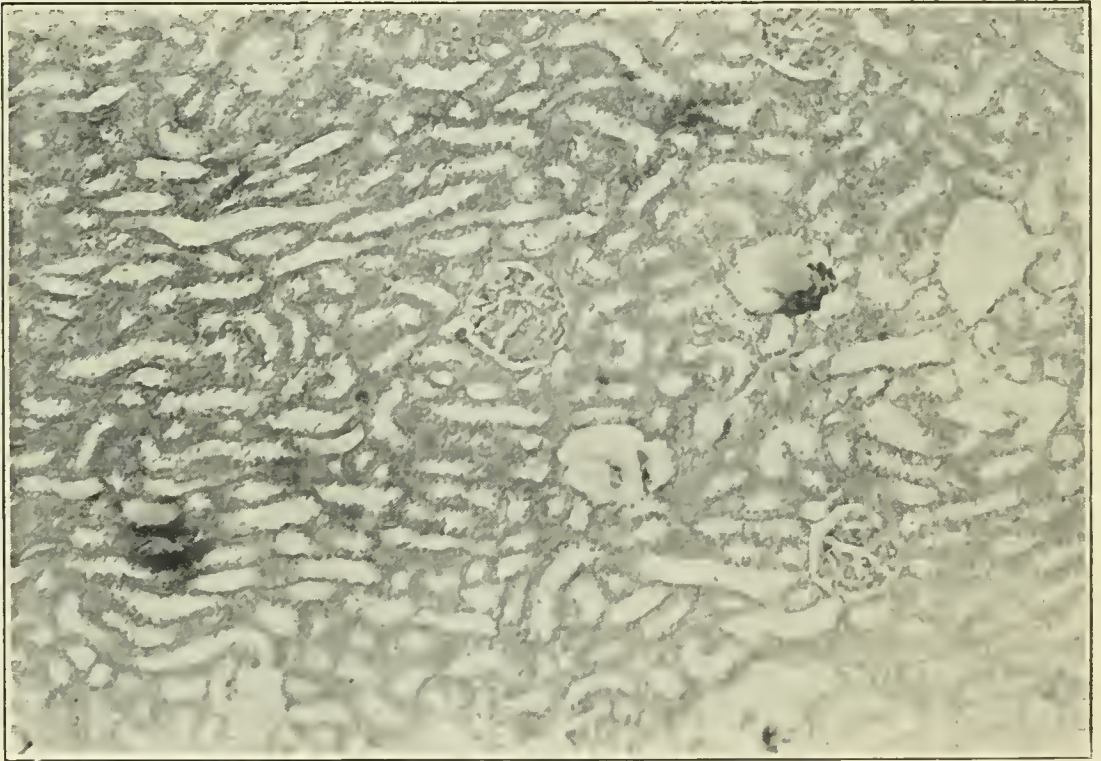


FIG. 2

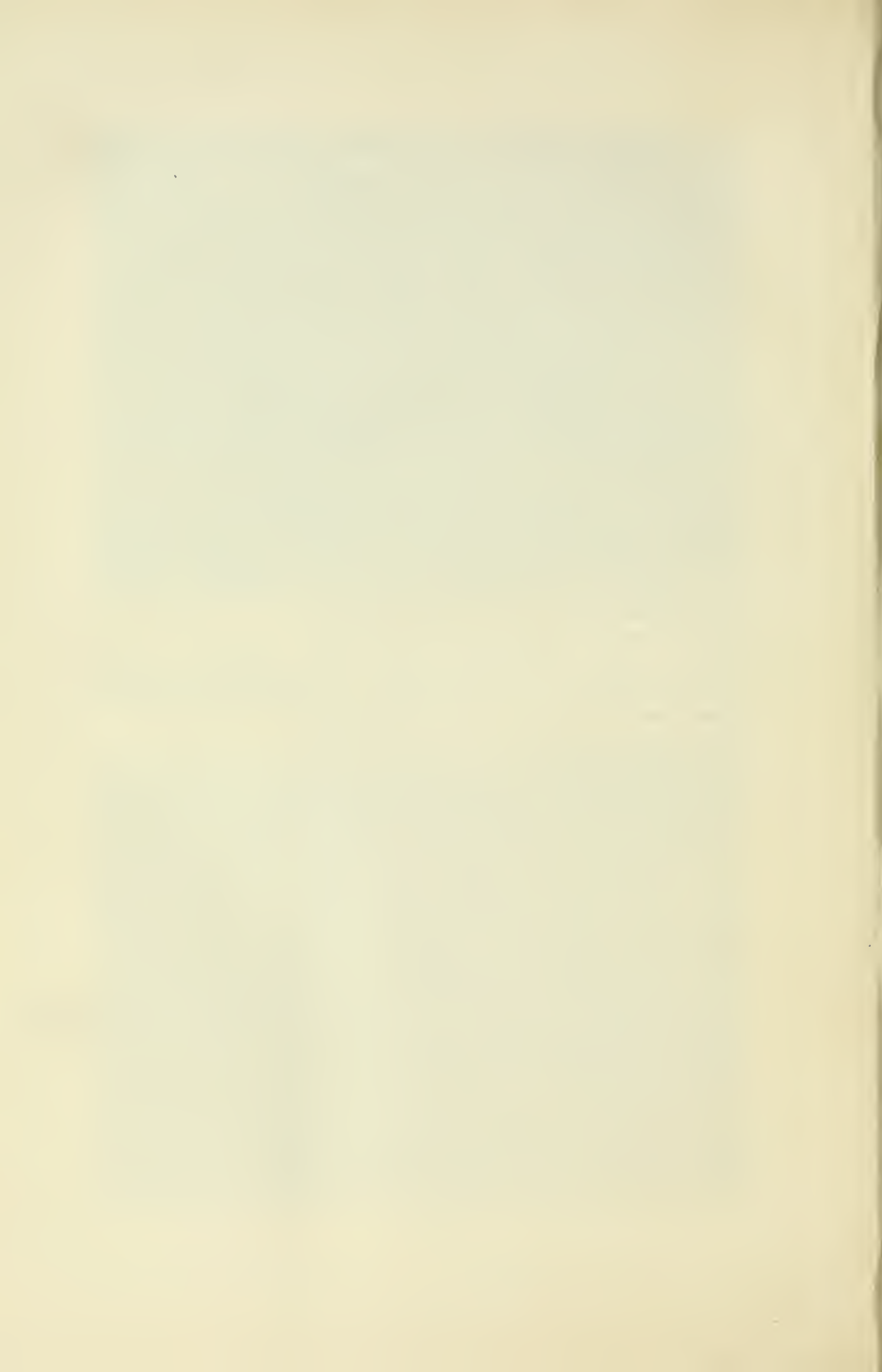
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#### EXPLANATION OF PLATE.

*Figure 1.* This shows the general shrunken appearance of all the glomeruli and destructive changes in a number of them. The possibility that the contracted appearance of the glomeruli is due in part to the anemia is not excluded.

*Figure 2.* This shows definitely destructive changes in a number of the glomeruli with practically no change from the normal appearance in a number of the other glomeruli in the case of a normal dog treated with germanium dioxide.





## METABOLISM IN ACETONEMIA OF MILCH COWS.

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In the last five years we have analysed several samples of urine and blood and also some samples of milk from milch cows suffering from a disease which is called acetonemia. In addition, we have studied the problem of the causation of this disorder.

This disease usually occurs about a week or ten days after parturition, sometimes somewhat later. It is especially observed in cows that give high milk yields and that are in excellent nutritional condition, i.e. more or less fat. The symptoms are: the odor of acetone, lack of appetite, growing worse as the disease advances, some obstruction and production of dry feces, decreased milk yield, and also forced breathing. A small number of the diseased animals are in an excited state. Insufflation of air in the udder is often used in treatment. The animals recover after some time, especially when put out to grass. It is an exception that they die from this disease, and in those cases there is probably a complication. There is a considerable loss to the farmer, because of the greatly reduced milk production.

We examined about twenty cases of acetonemia. Besides the samples from these, we analysed several other samples from diseased cows in which the urine contained less than 5 gm. acetone bodies per 1000 cc.; these cases therefore were at that time less serious. As the symptoms resemble those of indigestion, we wish to state here that acetonemia causes changes in the composition of the urine which are different from those due to acute indigestion. In some cases a veterinary made the diagnosis of acetonemia, but found he was mistaken when we declared the urine to indicate an acute indigestion.

## EXPERIMENTAL

In the urine we usually determined total acetone bodies, and several times also  $\beta$ -hydroxybutyric acid alone, total nitrogen, calcium, phosphoric acid, creatine, creatinine, the reaction to litmus paper and the specific gravity, also in several samples ammonia. We tested also with Fehling solution, but never found any sugar.

In the samples of blood we estimated cholesterol, fat, total acetone bodies, and sometimes  $\beta$ -hydroxybutyric acid, furthermore, in some samples the total lipoids (Bloor), the phosphoric acid present in the lipoids, glucose, the alkaline reserve and the non-protein nitrogen. In the milk samples we determined only total acetone bodies and fat, in some also the non-protein nitrogen.

As the composition of normal urine and blood of milch cows, especially shortly after parturition, is not very well known, we examined several samples from normal cows which were in that lactation period.

We tried to study the problem of the metabolic abnormalities in acetone-mia in two further ways. In the first place we fed cows, that had suffered in former years from acetonemia, during the last months before parturition more heavily than usual. The animals did not show the typical symptoms. The urines of these cows and others of the same farm, that were fed normally, furnished us valuable material for the study of the composition of normal urines.

In the second place we tried three times to provoke acetonemia experimentally, by producing glycosuria with injections of phlorizin followed by fasting. The urine contained 2.2 to 2.4 per cent glucose, but no acetone bodies during the days of the injections. Ketonuria occurred only when, after some days of phlorizin glycosuria, no food was given to the cows for two days. The quantity of acetone, however, was much lower than in typical acetonemia; about 0.4 gm. in 1000 cc.

Thus, neither fasting nor injection of phlorizin is sufficient to produce the typical disease which we are discussing. Even the combination of both gives rise to the output of only small quantities of acetone bodies. Therefore the cow does not easily produce much acetone. We found the same with a milch cow which suffered from diabetes, and gave urine with less than 1 gm. of acetone bodies in 1000 cc. Only severe disturbances of the metabolism, therefore, can give rise to the production of large quantities of acetone bodies in the organism of cows.

We must draw attention to the following additional points. It is not possible to determine the respiratory quotient of a cow in a simple way, as a cow cannot breathe through its mouth. A large respiration apparatus, in which the animal is put, is therefore indispensable for this purpose. It was also impossible for us to determine the volume of the urine excreted by the cows per day; nor could we analyse the mixed urines of 24 hours. Even if it had been otherwise, our figures would have had no absolute value, for with cows longer periods have to be taken. As we made several determinations in the same samples of urine, we knew whether they were of normal concentration and also whether the ratio of the different consti-



tuments was normal. Where we give figures for daily excretions, we always reckon on an excretion of 10 liters. The additional quantities of acetone eliminated in the breath were not determined. It is clear that our figures for different daily excretions can be considered only as approximate, still we believe that the errors are not such as to make our conclusions uncertain.

We estimate the caloric requirement of cows to be 12,500 per day, as our animals were cows of a normal weight of about 550 kg. This requirement was found by Kellner.<sup>1</sup> The expenditure of energy will be smaller than normal, as with continuance of the disease the food intake, and therefore the work of digestion, decreases. On the other hand these animals (being in the beginning of lactation) still always produce several kilograms of milk, sometimes as much as 8 kg. The caloric value of milk being about 700 for each kilogram, animals of 550 kg., that give more than 4 or 5 kg. milk and eat half a ration, require at least 12,500 calories.

#### METHODS.

For the determination of the acetone bodies, as well as for the alkaline reserve, we used van Slyke's methods.<sup>2</sup> We preferred not to filter the precipitate of the acetone bodies through paper, but to pour off the greater part of the liquid, to collect the precipitate in centrifugal tubes and to wash, dry and weigh in these tubes.

Phosphoric acid in the urine was estimated with uranum acetate using cochineal as indicator.

In some samples we made a check with the method of Bell and Doisy, modified by Briggs<sup>3</sup> or with the nephelometric method of Bloor<sup>4a</sup> altered by Kleinmann<sup>5</sup>.

For calcium we used either McCrudden's method<sup>6</sup> or that of Lyman<sup>7</sup>.

Fat was determined by the method of Leone-Lattes<sup>8</sup>. This method is a modification of that of Kumagawa and Suto. After saponification, rendering acid with hydrochloric acid, and, treating with ether the lipoids are finally extracted with petroleum ether. This is almost the same as Bloor's<sup>4b</sup> method of 1923 and seems to give about the same values. We usually found in the blood of normal milch cows (whole blood) 0.35 to 0.45%; sometimes lower or higher figures. Bloor gives as an average result 0.3%.

The phosphoric acid present in the lipoids, extracted by the method of Leone-Lattes, is much less than that in the total lipoids with Bloor's method of 1914-1918. Instead of about 0.044% we found in blood of three healthy milch cows less than 0.010%. We cannot decide the question whether the total lipoids of Bloor (1914) contain other phosphoric acid than that of phospholipins, or whether the greatest part of the phospholipins is not extracted by the method of Leone-Lattes (it may be that lecithin is hydrolysed and this makes the phosphoric acid insoluble in petroleum ether.)

We used the method of Grigaut for the determination of cholesterol (total, free and combined). This is a colorimetric method (green color with acetic anhydride and sulphuric acid). We compared this method with that of Bloor in some cases and found no great difference.

The total lipoids were determined by Bloor's method<sup>9</sup>, and the phosphoric

acid in the lipoids nephelometrically, as well as colorimetrically after Bell-Doisy-Briggs. To make the use of the last method possible we boiled with sulphuric acid and copper sulphate, adding the same amount of this salt to the standard solution.

Creatine and creatinine were estimated with Folin's picrate method. Also one of Folin's methods was used for the determination of ammonia, i.e., absorption with permutit and nesslerisation.

TABLE I.

Composition of urine and blood of milch-cows.

<i>Urine</i>	<i>Normal</i>	<i>In severe cases of acetonemia</i>
total acetone bodies	0.1-0.7 gm. in 1000 cc.	10-13 gm. in 1000 cc.
ammonia-nitrogen	0.0-20 mg. in 1000 cc.	about 500 mg. in 1000 cc.
calcium	about 40 mg. in 1000 cc.	" 800 " " 1000 "
phosphoric acid ( $P_2O_5$ )	about 40 mg. in 1000 cc.	upto 200 mg. in 1000 cc.
Glucose	absent	absent
reaction to litmus	alkaline	acid
<i>Blood.</i>		
total acetone bodies	traces	
cholesterol	0.1-0.12% (sometimes	0.8-1.0 gm. in 1000 cc.
total lipoids (Bloor)	0.65-1. 0% less)	0.2-0.24%
lipoid phosphoric acid	about 0.04%	0.5-0.7%
(Bloor) $H_3PO_4$		about 0.044%
lipoids (Leone-Lattes)	0.35-0.45 (usually)	0.3-0.4% (sometimes
alkaline reserve		less)
		4/5-3/5 of normal

Glucose was determined in the blood by Benedict's method with picrate solution.

#### DATA AND DISCUSSION.

The average figures of our analyses are given in Table I.

The urine of cows suffering severely from acetonemia contained from 10 to about 13 gm. total acetone bodies per 1000 cc.; the highest value was 13.6 gm. The amounts are like those found in human patients suffering from rather severe diabetes. As in the latter, about 4/5 to 2/3 of the total acetone bodies consisted of  $\beta$ -hydroxybutyric acid. Normal milch cows give in the first weeks after parturition urine that contains, according to our results,

less than 0.5 gm. total acetone bodies per 1000 cc. The urines of milch cows suffering from acetonemia very often contained more acetone bodies than nitrogen.

Instead of about 50 mg. Ca we often found more than 500 mg., sometimes 1000 mg. per 1000 cc. urine. Also the ammonia content was increased; the highest figures were about 500 mg. per 1000 cc. It seems therefore that the organism of the cow uses Ca to neutralize the acids ( $\beta$ -hydroxybutyric acid and acetoacetic acid) as well as ammonia. Several times the urine had an acid reaction, which is abnormal for the urine of the cow.

The amounts of phosphoric acid were usually slightly below 200 mg. instead of less than 50; therefore they were a little increased.

The amounts of creatine often surpassed those of creatinine. This we found also in some cases in urines of normal cows shortly, i.e. a few days or weeks, after parturition. The reason for this result is perhaps the decreased reduction of picric acid by creatinine in presence of acetone bodies, (i.e. the method was unsuitable in this case).

As stated already, glucose was never found in the urine of cows suffering from acetonemia. The blood of the diseased milch cows often contained 0.8 to 1.0 gm. total acetone bodies per 1000 cc. These amounts approximate those present in the blood of diabetic men.

As might be expected, the alkaline reserve was low in cases of severe acetonemia. The total amount of carbonic acid (after bringing the  $\text{CO}_2$  tension to the height of normal alveolar air) was  $4/5$  to  $3/5$  of the normal. The low values of the alkaline reserve are undoubtedly the reason for the abnormal respiration.

The glucose percentage in the blood may be considered normal. Our determinations, mostly made in 1919, were not very exact, since we used Benedict's (picrate) method, which, especially in the presence of acetone bodies, gives incorrect figures. The precipitation of the proteins with picric acid solution was always done in the freshly drawn blood. The determinations were finished within a few hours. We may conclude, at any rate, that there is in acetonemia no hyperglycemia of importance.

The non-protein nitrogen determinations gave normal results. The cholesterol content in normal blood plasma of cows we



found to be about 0.1 to 0.12% (sometimes less), in that of diseased cows about 0.17 to 0.25%.

The blood of cows suffering from acetonemia generally gives no high figures for lipoids by the method of Leone-Lattes. Perhaps we may say that there is a difference between blood of normal and of diseased cows, the percentage of lipoids (Leone-Lattes and Bloor) being lower in diseased animals; especially when they are convalescing (Leone-Lattes lipoids in normal blood usually 0.35 to 0.45; in blood of diseased cows 0.3 to 0.4%; total lipoids Bloor resp. 0.65 to 1.0% and 0.5 to 0.7%). As this blood is richer in cholesterol than normal blood, the difference being about 0.1%, the amount of other lipoids (lecithin and fatty acids) must be lower in the blood of the diseased animals. Our estimations of lipid phosphoric acid (Bloor) show no differences of importance; so it is probable that the phospholipins are present in about the same amount in both cases, and therefore the blood of cows suffering from acetonemia is poorer in fatty acids than normal blood, the difference being about 0.1 to 0.15%. The great tendency of the organism in acetonemia to catabolize the fatty acids may be the reason for these low amounts.

We analyzed the milk of the cows with typical acetonemia a few times and found 0.3 to 0.45% total acetone bodies. The content of lactose was sometimes low. Of interest was the very high percentage of fat, i.e. sometimes between 7 and 10%. The decrease of milk yield must be considered as the cause of these high figures. They are of importance in connection with the origin of the abnormal fat metabolism, for they prove that there was no milk fat resorption, so that the milk fat can not have been the source of the acetone bodies. Moreover, if we calculate that the daily output was about 120 gm. of acetone bodies and that for the production of this quantity about 330 gm. fat must be metabolised, it is clear that the fat from which the acetone bodies originated was not that of the milk.

The examination of urine and blood does not indicate an abnormal metabolism of the carbohydrates, and it shows that there was no lipemia. The only obvious abnormality in metabolism therefore is the formation of large quantities of acetone bodies. It is hardly possible to draw from this any other conclusion than the occurrence of a disturbance of the fat metabolism. Our cases therefore are instances of normal metabolism of carbohydrate

combined with abnormal fat catabolism. This proves that the two are regulated from different centers.

We analysed urine, blood, and liver of a cow that died 10 days after parturition, suffering from acetonemia. The urine and blood showed the same features as those of other diseased animals. There was some enlargement of the liver. It showed microscopically some fatty degeneration; notwithstanding this, there was not much increase in fat. We found 5.23 per cent. fat, and in three normal livers respectively 3.56, 2.55 and 5.5 per cent. The liver contained no glycogen. The liver tissue had a yellow color, probably due to bilirubin (we did not examine this point). The heart and kidneys of this cow showed the same degeneration as the liver. The fat of the heart had a yellow color.

It is probable that this animal did not suffer from acetonemia alone. The liver of the cow, examined three days after death, contained a very small amount of acetone, tested after distillation, with salicylic aldehyde and potassium hydroxide. Normal livers gave the same reaction, perhaps less distinctly.

In a dry cow, which was suffering from diabetes and which had about 3 to 4 per cent. sugar in the urine and 250 to 300 mg. of sugar in 100 cc. blood, determined by the methods of Folin and Wu and Shaffer and Hartmann, (normal milch cows usually have less than 100 mg. sugar in 100 cc. blood), the amount of acetone bodies in the urine was less than 1 gm. in 1000 cc. (twice we found 0.9 and about three months later (in May) 0.3 gm.) In urine of normal cows we found more than once 0.7 gm. of total acetone bodies. Acetone itself could not be detected with the salicylic aldehyde reaction.

The excreted sugar was evidently derived largely from the carbohydrate of the diet. We found a D: N. ratio of 5.8: 1 in January and one of 8.5 : 1 in May of the same year. Both samples contained 3.6 per cent. sugar; the first 0.625, the second 0.425 per cent. total nitrogen. These figures indicate, nevertheless, that the cow suffered from severe diabetes. She received no other food than hay (*ad libitum*) and 1 kg. linseed cake containing 10.85 per cent fat.

The content of total lipoids (Bloor) of the blood was 0.45%, the lipoid phosphoric acid about 0.04%, the cholesterol (in the plasma) 0.12%. Our analyses show that notwithstanding the disturbance of sugar catabolism, only a small amount of acetone bodies was excreted in the urine. This result seems to indicate that marked ketosis with glycosuria must be a very abnormal metabolic condition in cows.

Turning to our acetonemia cows, it may be supposed that they suffer from a severe functional disturbance of one of the organs concerned in the metabolism of fat. Fischler and others believe that experiments with the Eck fistula and the reversed Eck fistula have proved that the acetone bodies are formed in the liver. In line with this idea it may be mentioned that the livers of cows



shortly before parturition show a high fat content. An intoxication of the liver, of which the causative agent is produced by or in the organs connected with pregnancy or parturition, may be a plausible etiology of the acetonemia of milch cows. Reference may be made to experiments showing that the liver during pregnancy is less efficient than under ordinary conditions: e.g. Gottschalk<sup>13</sup> found a reduced tolerance for levulose and several times urobilinuria. Also a tendency to acetonuria during pregnancy in women has been observed. In connection with the hypercholesterolemia in acetonemia, we also refer to the experiments of Irene Barat<sup>14</sup>, who concludes that in man hypercholesterolemia always proves an abnormal functioning of liver cells, and believes that the liver provides for the normal cholesterol balance in the organism.

The fact that fat cows are most susceptible to the disease and that in the years 1917 and 1918, when there was less food than usual, the disease was rare, supports the view that heavy feeding provokes the disease in cows that are disposed to it. Fatness generally seems to conduce to metabolic disturbances. In this case the same may hold true, as in obesity the liver will probably be rich in fat and it (as well as other organs) may be able to produce large quantities of acetone bodies. As the disease lasts several days and a cow's liver weighs about 7 to 8 kg., of which about 3/4 to 4/5 is water, it is clear that not only fat from the liver but also other body-fat must be metabolized in order to account for the excretion of 120 gm. of acetone bodies per day. It is obvious that the experiments with the Eck fistula and the reversed Eck fistula (Fischler) do not prove that the acetone bodies are formed only in the liver. However, it is probable that the fat before being metabolized is transported to the liver. In acetonemia there is loss of appetite, and consequently a large breakdown of body fat. The intoxication seems to be the primary abnormal condition. The supposition that the ketosis results merely from complete or partial fasting is excluded by our observations mentioned above, and also by the further fact that in the urine of cows suffering from acute indigestion we found very large amounts of phosphoric acid, which do not occur in urine of acetonemia cows. We intend shortly to give the details of the abnormalities we found in urines of cows suffering from clinical or experimental indigestion. The acetonemia animals, however,



become very thin within a few days on account of the rapid consumption of body fat and protein. The large breakdown of tissue fat presumably diminishes when the supply is depleted. The animal begins to eat again and the symptoms disappear.

We wish to express here our thanks to Mr. H. Hooghoudt for his co-operation in our analytical work.

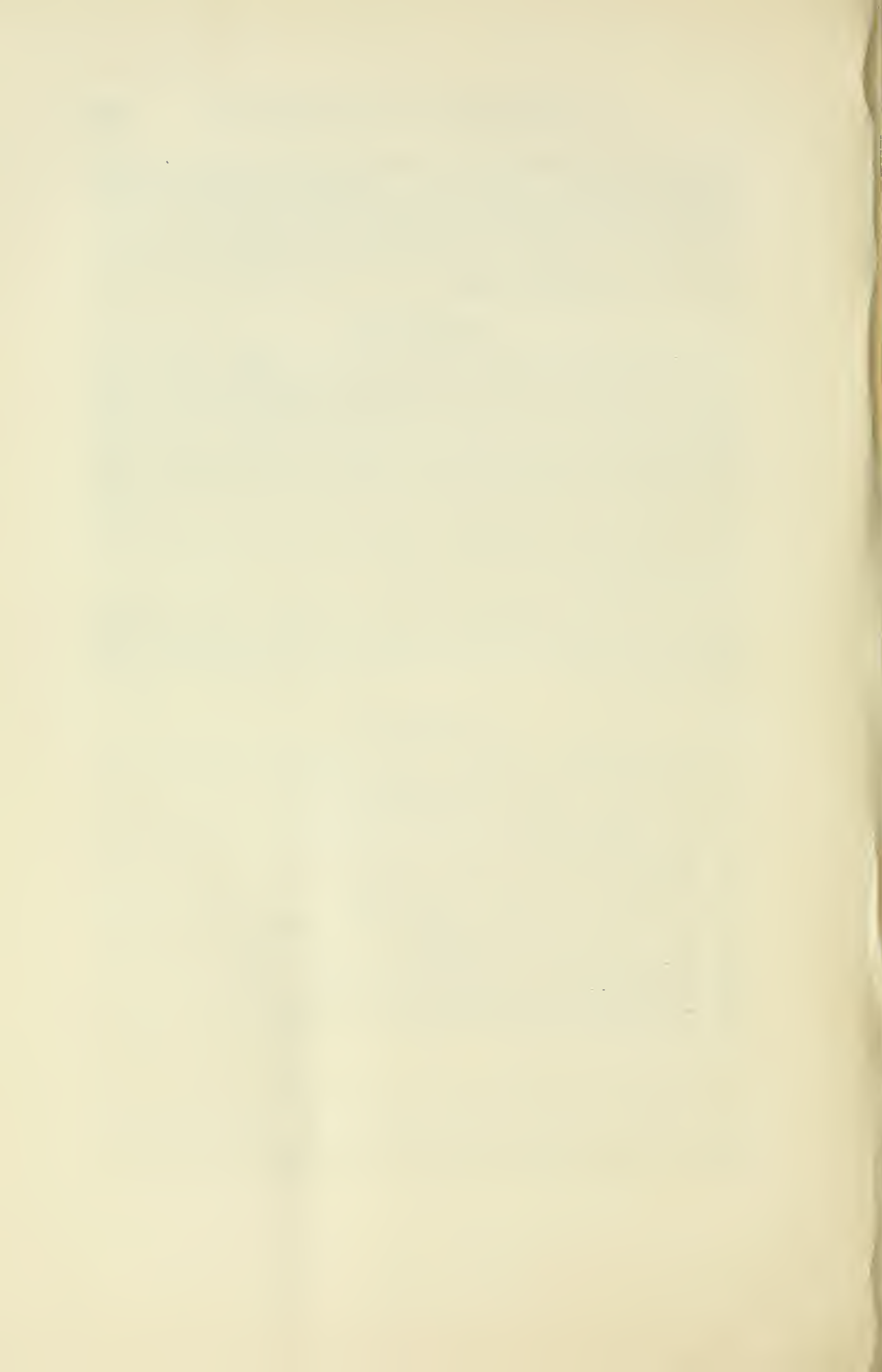
#### CONCLUSION.

In milch cows suffering from acetonemia shortly after parturition we found no evidences of disturbed sugar metabolism (glycosuria, hyperglycemia) and no lipemia. The lipid content of the blood plasma was normal or slightly below normal. The cholesterol showed an increase of about 100 per cent. The urine, blood and milk contained considerable percentages of the three acetone bodies. The amount of acetone bodies in the urine was in severe cases about 12 gm. per 1000 cc. The alkaline reserve of the blood was decreased.

The disease in question probably originates with functional changes of the liver caused by some intoxication, and the ketosis can then be explained by the abnormal catabolism of large quantities of body fat.

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# THE EFFECTS OF INTRAVENOUS INJECTION OF ANISOTONIC SOLUTIONS ON BLOOD CONCENTRATION

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Concentration of the blood has come to be recognized in certain diseased conditions as an important factor in the final outcome. Underhill and Ringer<sup>1</sup> state that "extreme blood concentration is incompatible with life and that if the blood concentration can be maintained within reasonable limits, the organism has a much better opportunity to combat the other toxic effects of the disease" (influenza). According to these workers, blood concentration means a failing circulation, an inefficient oxygen carrier, oxygen starvation of the tissues, fall of temperature and finally, suspension of vital activities. They recommend venesection and the introduction of fluid into the body, by way either of the mouth or vein. Underhill<sup>2</sup> considers the marked concentration of the blood in poisoning with the lethal war gases as immediately responsible for death and also observed this condition of the blood in the fatal cases following severe burns<sup>2a</sup>. Extreme dehydration takes place in the fatal cases of Asiatic cholera<sup>3</sup>. The effects of the extreme desiccation which takes place in the infantile dysenteriae, diarrhea and in inanition fever are well known.

Thus, the importance of therapeutic measures directed toward the prevention of this condition or its amelioration when it has ensued is apparent.

The hydremia following intravenous injection of isotonic and non-isotonic saline solutions has been observed experimentally by many investigators. Sherrington and Copeman<sup>4</sup> injected among other solutions water, physiological saline and rather strong hypertonic solution and determined the degree of dilution

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\* The data are taken from the thesis of Jacob B. Sigal, M. D., presented in partial fulfillment of the requirements for the degree of M. D., Yale University, 1923.



by means of the specific gravity of the blood. They concluded that water and physiological saline leave the circulation equally rapidly, which was contrary to the suggestion of Zuntz and Cohnstein<sup>5</sup> that the former left the circulation more rapidly; strong hypertonic solutions produce a greater and more lasting dilution. Boycott<sup>6</sup>, employing the hemoglobin method of the determination of blood dilution, concluded that in normal animals, physiological saline leaves the blood and passes into the tissues more rapidly than double this concentration of saline, and less rapidly than one-half the strength of physiological saline. More recently, Barach and his co-workers<sup>7</sup> have shown that after intravenous injection of strong hypertonic saline, the ensuing dilution lasts from three and one-half to four hours and corresponds to the isotonic equivalents of the salt injected. Bogert, Underhill and Mendel<sup>8</sup> concluded that "the regulation of the blood volume in normal animals is both rapid and efficient, for complete restoration of the original blood volume takes places within thirty minutes after the intravenous injection of a quantity of normal saline solution equal to the calculated blood volume of the animal."

Among the other effects observed in experimental animals following intravenous injection of non-isotonic solutions have been a diuresis and depression of the freezing point of the serum<sup>9</sup>, acceleration of the coagulability of the blood<sup>10</sup>, a rise in temperature, a rise in nitrogen and respiratory metabolism<sup>11</sup>, glycosuria and hyperglycemia<sup>12</sup> and variations in the cerebrospinal pressure<sup>13</sup>.

Therapeutically, intravenous injections of isotonic and non-isotonic saline solutions have been used more or less favorably in many conditions. Among them may be mentioned hemorrhage<sup>10</sup>, anaphylactic shock, bronchial asthma, bronchial catarrh, pulmonary congestion in anaphylactic shock<sup>14</sup>, severe toxic dysenteries, pulmonary edema<sup>14</sup>, neural syphilis<sup>15</sup>, cerebral herniae<sup>16</sup>, increased intracranial tension associated with internal hydrocephalus<sup>17</sup>. The strengths of the solutions used have varied from normal 0.9 per cent. saline to 15 per cent.

The present work is concerned with the effects on the normal blood concentration of the intravenous injection of weak hypo- and hypertonic sodium chloride solutions and the nature of some of the associated conditions.

## EXPERIMENTAL

The method employed in the following series of experiments consisted in the rapid intravenous injection of the saline solutions employed; and the determination of the hemoglobin of the blood taken before injection, immediately after and every five minutes for the first ten minutes and then every ten minutes to the end of an hour. Hemoglobin determinations were carried out according to the method advocated by Cohen and Smith<sup>18</sup>, all readings then being calculated on a one hundred per cent. hemoglobin basis to facilitate comparison of results.

Dogs in good nutritive condition and apparent good health were used in all experiments, they being, however, deprived of food and water for at least twenty-four hours previous to the experiment. The injection was made during ether anesthesia through a cannula into the femoral vein from a graduated cylinder at such a rate that the injection of the solution was complete in two minutes. Blood flowing freely from an ear vein was taken for hemoglobin determinations. Throughout the experiments 25 cc. of fluid per kilo of body weight (c.f. Bogert, Underhill and Mendel)<sup>9</sup> were injected.

The solutions employed in the majority of the experiments were 0.4 per cent. and 1.4 per cent. sodium chloride made up with distilled water and injected slightly warm.

Hemoglobin determinations are valid indices of blood concentration and dilution in the type of experiment described above. Smith and Mendel<sup>19</sup> state that anaesthesia, fright, withdrawal of successive small volumes of blood, and glycosuria observed following intravenous injections of saline, do not change the hemoglobin percentage. Bogert, Underhill and Mendel<sup>9</sup> have shown that the hemoglobin does not vary in amount in individual animals, at least, during periods of several hours duration and that a condition of food and water deprivation has little or no influence on the rate with which fluids leave the blood stream. Barach et alii<sup>7</sup> have shown that no hemolysis of red blood cells occurs as a result of the injection of hypertonic sodium chloride solutions.

THE EFFECT OF INTRAVENOUS INJECTIONS OF 0.4 PER CENT AND 1.4  
PER CENT NaCl SOLUTION ON THE BLOOD CONCENTRATION

When a quantity of weak hypotonic or hypertonic saline solution is injected rapidly into the circulation, the hemoglobin of the circulating blood is at once diminished, but this diminution of the hemoglobin is temporary and persists for a short time only; indeed the blood almost immediately begins to return toward its previous hemoglobin level.

There can be little doubt but that this is due to the speedy escape from the circulation of the saline injected. In illustration of the rapidity with which it is usual for this return toward the previous hemoglobin level to take place, the following experiments may be quoted:

From the above tables (I and II) it will be noted that the greatest dilution occurs immediately after injection and that

TABLE I

*Injection of 0.4 per cent. Sodium Chloride in 2 minutes.*

Weight kg.	Amount injected cc.	Hemoglobin before injection per cent.	Relative Hemoglobin after injection per cent.									
			Min. 0	5	10	15	20	30	40	50	60	

7.7	192.5	100	90	94	96	97	100	100	100	102	100
11.1	277.5	100	84	90	93	98	100	101	102	99	100
6.6	165.0	100	84	87	—	99	102	99	98	102	103
4.4	110.0	100	92	94	95	97	99	100	98	99	—
5.0	125.0	100	86	88	89	—	98	100	101	101	100
8.0	200.0	100	86	90	91	—	95	102	100	97	100
10.3	257.0	100	87	89	91	—	99	100	104	98	102

TABLE II

*Injection of 0.4 per cent. Sodium Chloride in 2 minutes.*

Weight kg.	Amount injected cc.	Hemoglobin before injection per cent.	Relative Hemoglobin after injection per cent.								
			Min.	0	5	10	20	30	40	50	60
5.6	140.0	100		72	84	90	92	93	100	99	102
8.7	217.5	100		83	87	92	94	98	100	100	100
10.2	255.0	100		84	90	91	92	93	97	100	100
9.2	230.0	100		82	88	95	96	99	102	100	100
11.8	295.0	100		78	88	89	93	96	100	101	103
5.6	140.0	100		77	84	87	93	95	100	102	100

within twenty minutes in the case of the hypo — and forty minutes in the case of the hypertonic solution the hemoglobin has returned to its original level; the rate of return in the majority of experiments was most rapid within five minutes after the injection. Furthermore, the initial decrease in hemoglobin is greater in the case of the latter than in that of the former, which is evidently an expression of the attempt on the part of the body to maintain the isotonicity of the blood on the one hand, and that of the tissue fluids by means of the factors of osmosis; in the case



of the hypertonic solution, tissue fluids pass into the circulation, in the case of the other, injected fluid passes out of the circulation into the tissues.

The rapidity of the action is noted by the fact that it is evident immediately after injection and must, therefore, take place during the injection.

Therefore, although quantitative sodium chloride determinations were not made along with those of hemoglobin, nevertheless these results suggest that the amount of salt injected plays a role in the rapidity with which fluids leave the circulation and in the degree of dilution. However, relatively, the rate of return is apparently more rapid in the case of the hypertonic solution, for although with the same relative amount more than three times the amount of salt was injected, nevertheless only twice the time was required to reach the original hemoglobin level.

It must be concluded, also, that the quantity of salts present in the circulation and tissues, the degree of fluid saturation of the body tissues at the time of injection, and the ability of the kidneys to excrete salts are factors determining the rapidity of rate of return and the degree of dilution.

Another possible factor determining the rapidity with which the fluids injected leave the circulation is suggested by the following experiments:

TABLE III

*Injection of 0.4 per cent. and 1.4 per cent. Sodium Chloride in 30 seconds.*

Weight kg.	Amount injected cc.	Hemoglobin before injection per cent.	Relative Hemoglobin after injection per cent.								
			Min.	0	5	10	20	30	40	50	60
5.5	137.5	100		82	87	100	98	98	96	102	103
8.3	207.5	100		80	86	94	96	100	98	98	103

It will be noted that when the injection time is diminished to thirty seconds, within ten minutes in the case of the hypo — and

within thirty minutes in the case of the hypertonic solution the hemoglobin has returned to its original level. Moreover, it will also be noted that the difference in the initial diminution in hemoglobin noted above is only slight and that the rate of return is most rapid in the second five minutes after injection.

Apparently, a preceding injection has no effect on the rate of return of a subsequent injection, as shown by the following which were made at an interval of fifteen minutes.

TABLE IV

*Injection of 1.4 per cent. Sodium Chloride with 15 minute interval.*

Weight kg.	Amount injected cc.	Hemoglobin before injection per cent.	Relative Hemoglobin after injection per cent.									
			Min. 0	5	10	15	20	30	40	50	60	
5.6	140	100	72	83	84	90	92	93	100	99	102	
	140	100	77	84	87	—	93	95	102	102	100	

In both cases the rate of return is most rapid in the first five minutes, although more rapid in the first than in the second injection.

### CONCLUSIONS

1. The regulation of the blood concentration in normal animals is markedly rapid and efficient, for when quantities of weak hypo — and hypertonic saline are injected intravenously it is found that the hemoglobin returns to its original level within twenty minutes in the case of the former and within forty minutes in the case of the latter.

2. Weak hypertonic saline is more efficient as a diluent of the blood than is weak hypotonic saline.

3. Taking into account the observations of Bogert, Underhill and Mendel mentioned above, weak hypertonic saline is somewhat more effective as a blood diluent than is isotonic saline.

4. A preceding injection does not affect the rate of return to the original hemoglobin level.

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## THE RATE OF ABSORPTION OF FLUIDS BY DIFFERENT ROUTES.—

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With the wider recognition of the significance of changes in blood concentration in various pathological conditions<sup>1</sup> has come a better appreciation of the value of fluid administration as a therapeutic aid in overcoming the detrimental effects due to this altered state of the blood. In general, experience has shown that for best results relatively large volumes of fluid administered for considerable periods of time are essential in order to accomplish this purpose.

At times under abnormal circumstances it becomes necessary or at least desirable to administer fluid by paths other than the alimentary tract. It is of distinct importance therefore to know the rate of absorption of fluid by the most obvious routes. For this purpose we have selected the rabbit as the experimental animal, since the quantity of fluid possible of administration by mouth may easily be exceeded owing to the size of the stomach, and in this way the limit of administration may be more or less accurately estimated and serve as a guide in the quantity of fluid to be employed parenterally. The investigation has included the administration of fluid (water, saline (0.9 per cent NaCl) and 5 per cent. dextrose solution), the routes chosen being by mouth, intraperitoneally and subcutaneously. The results demonstrate that there is no appreciable difference in the rate of absorption of fluid in the normal animal whether fluid is administered by mouth, subcutaneously, or intraperitoneally. On the basis of these facts one is justified in employing any or all of these paths

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\* The data are taken from the thesis of Hymen W. Weinstein, M. D., presented in partial fulfillment of the requirements for the degree of M. D., Yale University, 1923.

of absorption in abnormal conditions in which it is desired to alter blood concentration.

#### EXPERIMENTAL

*Methods.* — Normal full-grown rabbits were maintained in a fasting condition for 24 hours previous to experimentation, although water was allowed. The rate of absorption of fluid was followed by hemoglobin estimations, the method of Cohen and Smith being employed with blood obtained from the marginal ear vein. Fluids were given (a) by mouth, (b) subcutaneously, (c) intraperitoneally, the fluids being water, saline (0.9 per cent. sodium chloride) and glucose (5 per cent. solution). When fluids were given other than by mouth they were sterilized and administered under aseptic precautions. Other details may be found in the tables appended.

#### THE RATE OF ABSORPTION OF FLUID BY DIFFERENT ROUTES.

The accompanying tables (Tables 1 to 9), which are merely illustrative of many similar experiments, are clearly uniform in the results obtained. They demonstrate that irrespective of the path of absorption or of the fluid administered, even though relatively large volumes are given, there is no appreciable difference in the rate of absorption of the fluid when employing changes in hemoglobin content as a criterion of fluid alterations in the blood.

#### CONCLUSIONS.

There is no appreciable difference in the rate of absorption of fluid whether administered by mouth, subcutaneously, or intraperitoneally as determined under the experimental conditions employed.

This fact carries with it justification for the employment of any or all possible paths of absorption when it is desired to introduce fluid into the body in order to restore to the normal level concentrated blood.



TABLE 1

*The Influence of Oral Administration of Water upon Hemoglobin Values*

Time	Water Intake cc.	Hemoglobin in percentage of normal
5:10	50	100
5:30	50	—
5:35	—	101
5:50	50	—
6:10	50	—
6:15	—	99
6:30	—	100
7:00	—	99
7:45	—	99

TABLE 2

*The Influence of Subcutaneous Administration of Water upon Hemoglobin Values*

Time	Water Intake cc.	Hemoglobin in percentage of normal
9:15	50	100
9:35	50	—
9:40	—	100
9:55	50	—
10:15	50	—
10:20	—	102
10:35	—	102
11:05	—	101
11:50	—	101

TABLE 3

*The Influence of Intraperitoneal Administration of Water upon Hemoglobin Values*

Time	Water Intake cc.	Hemoglobin in percentage of normal
2:50	50	100
3:10	50	—
3:15	—	99
3:30	50	—
3:50	50	—
3:55	—	100
4:10	—	99
4:40	—	99
5:25	—	99

TABLE 4

*The Influence of Oral Administration of Saline (0.9 per cent) upon Hemoglobin Values*

Time	Water Intake cc.	Hemoglobin in percentage of normal
9:45	50	100
10:05	50	—
10:10	—	99
10:25	50	—
10:45	50	—
10:50	—	97
11:05	—	98
11:35	—	98
12:20	—	100

TABLE 5

*The Influence of Subcutaneous Administration of Saline (0.9 per cent.) upon Hemoglobin Values*

Time	Water Intake cc.	Hemoglobin in percentage of normal
10:10	50	100
10:30	50	—
10:35	—	102
10:50	50	—
11:10	50	—
11:15	—	101
11:30	—	101
12:00	—	99
12:45	—	102

TABLE 6

*The Influence of Intraperitoneal Administration of Saline  
(0.9 per cent.) upon Hemoglobin Values*

Time	Water Intake cc.	Hemoglobin in percentage of normal
9:40	50	100
10:00	50	—
10:05	—	101
10:20	50	—
10:40	50	—
10:45	—	100
11:00	—	100
11:30	—	101
12:15	—	101

TABLE 7

*The Influence of Oral Administration of Glucose (5 per cent.)  
upon Hemoglobin Values*

Time	Water Intake cc.	Hemoglobin in percentage of normal
9:55	50	100
10:15	50	—
10:20	—	98
10:35	50	—
10:55	50	—
11:00	—	97
11:15	—	100
11:45	—	101
12:30	—	100



TABLE 8

*The Influence of Subcutaneous Administration of Glucose  
(5 per cent.) upon Hemoglobin Values*

Time	Water Intake cc.	Hemoglobin in percentage of normal
4:55	50	100
5:15	50	—
5:20	—	103
5:35	50	—
5:55	50	—
6:00	—	100
6:15	—	97
6:45	—	100
7:30	—	101

TABLE 9

*The Influence of Intraperitoneal Administration of Glucose  
(5 per cent.) upon Hemoglobin Values*

Time	Water Intake cc.	Hemoglobin in percentage of normal
9:15	50	100
9:35	50	—
9:40	—	101
9:55	50	—
10:15	50	—
10:20	—	101
10:35	—	99
11:05	—	99
11:50	—	101

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## THE UTILIZATION OF FAT IN THE ANIMAL BODY\*

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Attention has recently been sharply focussed on fat and its intermediary metabolism because of the controversy regarding the use of high fat diets in diabetes. It would be premature to enter into the merits of the discussion at the present time since the subject is in the stage of rapid development with new facts and conceptions appearing with each new publication. But the differences of opinion and the resulting experimentation are of the greatest importance in the clarification of our ideas on intermediary fat metabolism and it has seemed to me therefore a particularly good time to review our knowledge and determine just where we stand in this interesting but difficult field.

*The place of fat among the foodstuffs.* — Ordinarily fat supplies about one-fifth of the caloric requirement. This percentage is increased with increased energy requirement, and also in diabetes owing to the failure to burn carbohydrates. Fat is the most concentrated foodstuff and the only one stored in large amounts. It is not water soluble and probably for this reason is the most difficultly digestible of the foodstuffs. Burned alone or in large proportion it is not an economical fuel, Krogh and Lindhard<sup>1</sup> having shown that the waste of energy in work done on fat as compared with carbohydrates is about 10 per cent., and in addition that the fatigue induced by the work is considerable and often excessive with fat as compared with carbohydrate (acidosis excluded). When fat supplies over 80 per cent. of the energy requirement, the factor of incomplete combustion with production of acetone body acids becomes a source of danger.

*Chemical Nature and Relations.* — The fats are compounds of the higher fatty acids with glycerol. For purposes of this study

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\* Lecture before the Harvey Society, New York, November 3, 1923.

they may be considered simply as fatty acids, the glycerol component playing a relatively insignificant part. The fatty acids ordinarily found in food fats are sixteen and eighteen carbon-atom straight-chain compounds, none of them highly unsaturated. The exception is the important fat, butter, in which there is a notable proportion of the short-chain fatty acids. In the consideration of the utilization of fats it is becoming increasingly necessary to take into account various other compounds of fatty acids in the organism, substances which are ordinarily classified as lipoids, among which are the various compounds of which lecithin is a type — compounds of the fats with phosphoric acid. The part taken by phosphoric acid in the metabolism of the carbohydrates is already well recognized. The importance of phosphoric acid combined as lecithin, in the utilization of the fatty acids, is supported by a good deal of data to which reference will be made later. The complex aromatic alcohol cholesterol appears also to be closely connected with the metabolism of the fatty acids through its fatty acid esters. Lipoids of other types — compounds of fatty acids with carbohydrate radicles, etc. — are largely confined to the brain and nerve tissue and their presence there gives a basis for some interesting speculation because of the close relationship found to exist between the fatty acids and the carbohydrates in metabolism.

The fact that these various substances are compounds of fatty acids and undoubtedly concerned in their metabolism, indicates that a separate classification into fats and lipoids is indefensible and that both should be grouped together as phases of their common metabolism. A classification embodying these ideas and with the fatty acid as the basis of classification was published a year or two ago<sup>2</sup>.

It appears possible also to divide the fatty acids of warm blooded animals into two functional groups according to whether or not they are in immediate use in the body, as Active and Inactive Fatty Acids. The division for warm blooded animals may somewhat arbitrarily be made as follows: Those acids more unsaturated than oleic acid are classed as active, all others including oleic and the saturated acids as inactive. The main reason for this division is that it is rare to find in the stored and therefore inactive fat of warm blooded animals, any acid less saturated than oleic, while the fatty acids of the tissues which are continu-



ously active, such as heart, kidney and brain, are largely of the more highly unsaturated type. The classification does not apply to the fatty acids of cold blooded animals nor of plants, for the fatty acids found in the fat stores of these organisms are often highly unsaturated. The reason for the difference appears to be that a stored fat in any organism has to satisfy two requirements: (1) it must be stored in such a form as to be available i.e. liquid, at any temperature to which the living organism may be submitted, which means that in cold blooded animals and plants there must be a considerable proportion of the highly unsaturated (low-melting) fats present, and (2) the stored fat must not be spontaneously oxidizable at the environmental temperatures, which necessarily limits the amount of the highly unsaturated fat present. In warm blooded animals where the body temperature is nearly constant and relatively high, a suitable admixture of olein with the saturated fats is sufficient to preserve fluidity.

*The Metabolism of the Fats.* — Certain phases of the subject have already been dealt with in lectures before the Society — the function of the liver in fat metabolism by Leathes, the rôle of fat in diabetes by Allen, and the balance between the carbohydrates and fat in fat combustion by Shaffer, and for that reason most parts of the subjects covered by them may be passed over rather briefly. Also, only a brief treatment will be given to the processes of digestion, absorption and transport, since very little that is new can be said about them and since it seemed desirable to spend most of the time in a discussion of the intermediary metabolism of the fatty acids. Furthermore, since the time available is short, it seems wise to pass over with brief reference certain phases of the subject which in a discussion of this kind might well be given major mention but of which excellent reviews are available.

*The Digestion and Absorption of the Fats.* — The peculiarities in the behavior of fats in digestion and absorption may be referred largely to the fact that they are insoluble in water. Because of this insolubility the changes in the stomach are ordinarily slight, although a lipase is present. Only when fat is present in emulsified form (as, for example, milk) is there any notable amount of fat digestion in the stomach. Their insolubility may explain the fact that they inhibit or perhaps rather do not

stimulate gastric activity and consequently are slow in leaving the stomach. So far as is known no absorption takes place in the stomach.

In the intestine, fats being insoluble in water, while the lipases are soluble, digestive action takes place only at the fat-water interfaces and is therefore effective only when the surface is greatly increased, as by emulsification. The main emulsifying agents are the soaps — the water soluble form of the fatty acids, — but only second in importance is the bile, which appears to have been provided especially for the digestion of the fats, since it not only aids in forming and preserving the emulsion but by means of its salts dissolves large amounts of the fatty acids and is a very important factor in their passage through the intestinal wall. In the absence of bile very little fat absorption takes place. The form of absorption is uncertain but presumably is as soaps or fatty acids, for the facilities for splitting the fats are so adequate that only traces of unsplit fat are found in the feces. If so, there is immediate synthesis in the intestinal wall (a marked difference from the other foodstuffs), for the absorbed material leaves the intestine as fat. The entrance into the blood stream of the absorbed fat takes place apparently only by way of the lymphatic system and it is thus not submitted like the other foodstuffs to the censorship of the liver, useless or harmful substances apparently being stopped at the port of entry in the intestine. Thus the fats are transferred to the tissues from the food bodily, and not furnished to the cells in the form of building stones as is the case with the other foodstuffs.

The region of absorption of the fats in the intestine is apparently more limited than that of the other foods. There is no absorption from the large intestine, and fat not quickly absorbed in the small intestine may escape altogether, as shown by the fact that large amounts of fatty acids may reach the large intestine and the feces, indicating a failure in absorption and not in digestion. A probable explanation of the failure of absorption may be found in the fact that the bile salts are absorbed from the intestine to be used over again (circulation of bile), and since these are known to be very important<sup>3</sup> for fatty acid absorption, any fatty acids left unabsorbed after the absorption of the bile would be wasted.

The fats thus differ fundamentally from the other foodstuffs



in their manner of digestion and passage from the intestine to the blood stream. All evidence points to their complete splitting into fatty acids and glycerol and their absorption as soaps, i.e. their water soluble form, but the soaps because of their large molecule and their ready hydrolysis and powers of aggregation probably do not behave as crystalloids but rather as colloids with fairly large particles, so that the splitting of the fats probably does not greatly simplify their handling during absorption, hence the great importance of the solvent powers of the bile acids.

An entirely separate channel is provided for the conveyance of fats to the blood. They reach the circulation by way of the lymph vessels and apparently do not pass in any appreciable amounts into the intestinal blood system. The most recent efforts to demonstrate their increased presence there<sup>4</sup> failed to disclose any definite difference in the fat content of the jugular, portal or mesenteric veins. However, the methods used were not exact enough to show small differences which might be significant. Some older work by Joannovics and Pick<sup>5</sup> has a bearing on this point but only serves to complicate the situation. Their experiments were as follows: Dogs were fed with oil having an iodine number of 120-130 and their liver fat examined at the height of digestion. It was found that the fat of the liver had doubled in amount (as compared with normal animals) and had an iodine number higher than the fat fed and much higher than the liver fat of animals on ordinary food, indicating an accumulation of fat in the liver during digestion and also a desaturation. In dogs with an Eck fistula there was no rise of liver fat nor increase of iodine number, indicating that normally the portal vein is an important factor in the accumulation of fat in the liver.

As far as our present information shows, the fragments of the other foodstuffs produced by digestion reach the blood directly as such without synthesis in the intestinal walls. The fatty acids and glycerol are built up into fats again before they leave the intestinal walls, passing out from the epithelial cells and across the lumen of the villus to the openings of the lymph system in a manner the details of which are largely unknown. The reason for the synthesis and thereby the difference from other foodstuffs may probably be sought in the fact that soaps in the blood stream would be dangerous, due to their hydrolysis and consequent alkalinity and to the known hemolytic effect of some of them. The



absorbed fat reaches the blood stream by the lymphatic system and mainly through the thoracic duct, while none of the other foodstuffs enter by that way. In view of the similarity in fat content of the portal and general circulation, the fact that only about 60 per cent. of the absorbed fat can be recovered from the thoracic duct at its point of entry into the blood system was a considerable stumbling block until quite recently when Lee<sup>6</sup> was able to show that the thoracic duct is probably not the only opening by which the absorbed fat reaches the blood stream.

The synthesis of fat during the passage through the intestinal cells gives an opportunity for certain adaptive changes. The possible changes may be (a) a rearrangement of the fatty acids attached to the glycerol molecule, producing mixed glycerides from simple ones or vice versa; (b) a saturation or desaturation of the fatty acids with corresponding change of properties; (c) a dilution with fat from nearby depôts especially the liver; (d) a selection at different stages of digestion of certain of the various fatty acids set free by hydrolysis. Under suitable conditions changes due to one or more of these causes have been shown to take place. For example, after feeding a high melting saturated fatty acid such as stearic acid, the chyle contains considerable amounts of low-melting unsaturated fat<sup>7</sup>. And when moderate amounts of triolein — an unsaturated, low-melting fat — is fed the fat of the chyle contains measurable amounts of saturated high-melting fat. The mechanism appears only moderately effective, for it is well known that when a large amount of fat is fed it is transferred to the fat depôts without demonstrable change. The fact should be pointed out, however, that under normal conditions it is rare for fat enough to be taken with the diet to produce any definite effect on the fat of the depôts, each animal storing a fat which is characteristic of the species and which probably represents a balance between utilization and storage of the food fat and of the fat synthesized from carbohydrate. The fat which reaches the blood, although originating in the food fat, may thus under suitable conditions have quite different properties, and the part taken by the intestinal wall in the modification of the fat passing through it is worthy of further investigation.

The fat enters the blood stream in the form of very finely divided particles (variously called "fat dust," hemakonia, chylo-

microns,) about  $1\ \mu$  in diameter and with a pronounced Brownian movement. At the time when the fat reaches the blood or soon after there is to be noted an increase of lecithin (lipoid phosphorus) and sometimes cholesterol<sup>s</sup> in the blood, the former of which is believed to have its origin in the fat and to be a stage in its utilization. The advantage of a change from fat to lecithin in the blood is easily seen. The blood lecithin is to all intents and purposes water soluble and the greater ease of handling such a substance in a watery system is apparent. The close chemical relationship of lecithin to fat makes the change relatively simple and the greater sensitiveness of lecithin to chemical change opens a path for the later series of metabolic changes. The advantage of an increase of cholesterol in the blood is not so apparent, since although it forms esters with the fatty acids these are chemically more inert and if anything less soluble than the fats.

Nothing definite is known regarding the transference of the blood fat to the tissues, but the indications are that there is more than one way by which it is accomplished. During the time of high fat content of the blood, fat is found collected at various points of the endothelial lining of the blood vessels, and it is probable that certain of the cells have the power of engulfing fat particles that come within their reach and that they can pass them on into the tissues.

Attention should be called at this point to the ability possessed by many living cells to reduce fat to a form so finely divided as to be invisible in the microscope, therefore presumably colloidal. Also there can be no question but that the transformation of fat into the water soluble lecithin is of great importance in its passage from the blood, as Meigs and his co-workers<sup>10</sup> have shown for milk fat secretion.

Nor can anything definite be said of the passage of fat out from the tissue cells into the blood when needed for combustion, except that it is not a simple process. Under most conditions the discharge from the fat tissues into the blood is not rapid enough to produce any marked changes in the lipoid composition of the plasma, but when the storage cells are filled to capacity the sti-

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\* From the clinical point of view it is worth noting that there appears to be a parallelism between the fat content of the blood as determined by a count of the visible particles<sup>9</sup> and the content determined by chemical methods, indicating that the method of determination by count may have considerable clinical value in the study of fat absorption in the intestine.



mulus to empty may cause an excessive discharge sufficient to produce not only an increase in lecithin and cholesterol but also a visible lipemia. This excessive discharge appears to depend on local instability of the fat stores, since the general fatness or leanness of the animal is apparently not an important factor.<sup>11</sup>

When not disturbed by excessive inflow of fat, the blood lipoids of both corpuscles and plasma bear a constant relation to each other. According to our present information there is little if any true fat (glycerides) to be found under these conditions in either plasma or corpuscles, cholesterol with its fatty acid esters (in the corpuscles, cholesterol alone) and lecithin (phospholipoid) making up the lipid content. All available data indicate that there is a constant balance between cholesterol and its esters on the one hand and cholesterol and lecithin on the other. The fact of a similar balance in most tissues has been established by the French workers in this field<sup>12</sup>. The balance between the lipid constituents of blood is closely maintained unless the increase in lipoids is very great, when there may be a disproportion. Thus when there is a large and sudden increase of lipoids, as sometimes happens in diabetic lipemia and in the hemorrhagic lipemia of rabbits, the first constituent to increase is the fat, next the lecithin, and latest the cholesterol which generally continues to increase after the increases in lecithin have ceased, exhibiting thus the same sequence as is found during alimentary lipemia<sup>13</sup>. After the lipemia is established the cholesterol and lecithin bear about the same relation to each other as in fasting blood. Even in the severe lipemia of diabetes and in the hemorrhagic lipemia of rabbits with a large increase of total lipid this balance is pretty well preserved<sup>14</sup>, which is in accord with the general conception of the balance of composition of the blood. The blood corpuscles are apparently not concerned in any but the alimentary lipemia, since in the persistent lipemias their lipid constant is about the same as in fasting blood.

*Intermediary Metabolism.* — Regarding the intermediary metabolism of the fats, our present conceptions spring from the work of Leathes who embodied his researches and generalizations in a lecture before this Society in 1909. Put briefly, his belief is that the fats intended for immediate use, whether from the food or from the fat depôts, are carried to the liver where the fatty acids undergo the first stages in their breakdown, which consist



in the introduction of double bonds at various points in the long chain. These points thereby become points of weakness which are first attacked by oxidation with the result that the long chain is broken, yielding a series of fragments which are then disposed of separately. Leathes believed also that a transformation of the fat to lecithin may take place in the liver and that the desaturated, phosphorized fats are then distributed by the blood to the tissues for utilization. Since Leathes' lecture considerable material has accumulated which has a direct bearing on his hypothesis and it is desirable to consider the applications of this new work to his ideas.

The work of Leathes and his fellow workers regarding the fact of desaturation can, I think, be accepted without question. Also there is no doubt that the liver has marked power of desaturation and that it is the most important desaturating organ. Whether other organs are concerned in desaturation is uncertain since some of them such as the heart, kidney, brain, contain highly unsaturated fatty acids and during fat mobilization their fat content increases somewhat, although to nothing like the extent of the liver. If the liver is to be regarded as the sole or main place of desaturation from which the unsaturated fatty acids are carried by the blood to the tissues for use, then it is necessary to demonstrate the presence of highly unsaturated fatty acids in the blood, which it has been possible to do in recent work<sup>15</sup>. The liquid fatty acids of the fasting blood plasma of various animals were found to have iodine numbers up to 160 and averaging in beef blood 147, dog 155, pig 153 and sheep 118, numbers which indicate the presence in considerable amounts of fatty acids with at least two double bonds and, as was shown by the behavior of the acid mixture, probably some with more than two.

The second part of Leathes' hypothesis, the conception that lecithin is formed from fat in the liver as a stage in the utilization of fat and as the carrier of the unsaturated fatty acids from the liver to the tissues where they are to be utilized, is not quite so readily acceptable in all its details. The idea of lecithin as an intermediate stage in certain phases of fat utilization as noted above is attractive and highly plausible. In addition to the finding of Leathes and others that lecithin is found in the liver, heart, kidney, muscles, brain, etc., where fat metabolism is ordinarily

active, it is found to increase in the blood and particularly in the corpuscles during the absorption of fat and in certain disorders of fat metabolism where it keeps pace with increases in other lipoids. The researches of Meigs<sup>10</sup> and his co-workers make it extremely probable that blood lecithin is the mother substance of milk fat. Its close chemical relationship to the fats adds further weight to the belief, since it may be regarded as a phosphorized fat — a glyceride in which one of the fatty acids is replaced by a phosphoric acid residue. The resulting compound, in contrast to the fats, mixes readily with water to form a homogeneous mixture which in the case of freshly prepared blood lecithin is almost clear, forming thus a water-soluble vehicle for the insoluble fats which must be of great significance in the utilization in a watery medium such as the animal body. In this connection also the fact that lecithin is an excellent emulsifier must be of great importance in preserving the solution in the blood of such insoluble substances as cholesterol and the cholesterol esters. Lecithin is much more active chemically than the fats. It is more readily hydrolyzed and more readily oxidized, so much so, indeed, that it is very difficult to prepare it in pure form for examination. Enzymes are present in blood and tissues which hydrolyze lecithin readily but attack the fats hardly at all<sup>16</sup>. Our information regarding the tissue lecithins is becoming increasingly definite and accurate due to the efforts of Levene<sup>17</sup> and MacLean<sup>18</sup>, and it is found that the two fatty acids in combination in tissue lecithins are generally different and generally one saturated and one unsaturated. According to the most recent reliable work (Levene) the fatty acids ordinarily in combination in lecithin and the closely related cephalin are often of a considerable degree of unsaturation, four double bonds being reported.

The tissue lecithins are optically active, which fits in well with the belief that optical activity is closely related to the phenomena of life. Another property of the lecithins, which increases their importance in the consideration of conditions in the living cell, is their power of forming loose combinations with all sorts of

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\* In this connection attention is called to the significant fact reported by Levene<sup>19</sup> that the highly unsaturated fatty acids of these lecithins and cephalins are split out by cobra venom, leaving a residue containing only saturated fatty acids (lyso-lecithins and lyso-cephalins), indicating that the unsaturated acid is more readily removed than the saturated acid. The relation of this finding to the transport of the unsaturated fatty acids by lecithin is evident.



substances, sugars, proteins and salts, a property which may well be valuable in preserving the unity of the protoplasm of living tissue. The lecithins are thus well suited for the part which they are believed to play in the transport and the initial steps in the intermediary metabolism of the fatty acids. By the substitution of a phosphoric acid residue for one fatty acid the fats become at once reactive and water soluble, ready to participate in all the changes of a watery medium. The idea that the desaturated fatty acids are formed into lecithin in the liver and as such are transported by the blood to the tissues is based on the fact that lecithins containing highly unsaturated fatty acids are to be found in the liver and also in the heart, kidney, and other organs and tissues. If they are transported in the blood, lecithins containing highly unsaturated fatty acids should be demonstrable there. Investigations on this point now in progress in our laboratory do not give an entirely satisfactory answer although they do not exclude the possibility. Iodine numbers of the fatty acids of the lecithin fraction of plasma lipoids are found to run generally between 80 and 90 with a few as low as 40 and an occasional one over 100. The fatty acids from the lecithin fraction are a mixture containing both solid and liquid acids. The exact amounts of each have not been determined nor is it possible by the method used to extract the lecithin without partially destroying it. The results so far indicate that some of the blood lecithins are of the same type as the liver lecithin recently described by Levene<sup>17a</sup>, containing one saturated and one unsaturated fatty acid with an iodine number of from 160 to 180 and containing therefore probably two double bonds. The data are made more difficult of interpretation by the fact that in blood in the fasting condition, transport of saturated fatty acids from the fat depôts to the liver must be going on simultaneously with transport of the unsaturated fatty acids from the liver to the active tissues. The findings so far are, however, not incompatible with Leathes' hypothesis regarding unsaturated acid transport.

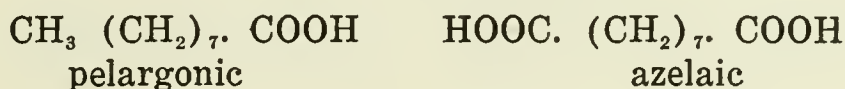
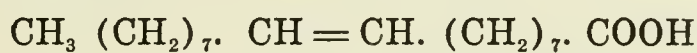
In connection with the mode of transport of the unsaturated fatty acids in blood, mention should be made of work now under way which has an important bearing on the function of cholesterol in fatty acid metabolism, which brings us again to the dead wall of our ignorance as to the significance of this mysterious substance. Cholesterol is universal in its occurrence in tissues



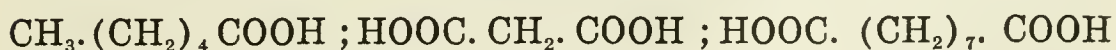
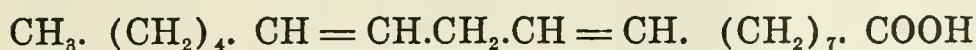
wherever there are other lipoids, sometimes free and sometimes in the form of esters of the fatty acids. It is a relatively stable substance and there is no satisfactory evidence that it is burned in the body. It originates mainly in similar substances in the foods and, as with the sodium chloride of the blood, there is ordinarily a constant flow of it through the organism, in with the food and out in the feces by way of the bile. In the blood when not disturbed by food absorption it bears a constant relation to lecithin and in the plasma to its own esters. In the corpuscles it is always found free. In the blood plasma it increases as the other lipoids increase, and any increase of cholesterol due to overfeeding is followed by an increase in the other lipoids<sup>20</sup>. It is an insoluble substance and yet can be carried in apparent solution in the blood in amounts up to nearly two per cent., as in severe diabetes. It, or one of its slightly changed forms, is the main constituent of the fatty material of the skin secretions and finds its usefulness there largely because of its great stability under conditions where oxidation would be very easy — thin layers and exposure to light and warmth. When fed in relatively large amounts there is some evidence<sup>21</sup> that it or its esters may deposit in the tissues and in the walls of the blood vessels. Its tendency to deposit in the gall bladder is too well known to call for comment, and some writers have found a relation between high blood cholesterol and cholelithiasis<sup>22</sup>. In blood plasma it occurs both free and combined in the proportion of about one part free to two parts combined. From the work of Hürthle<sup>23</sup> the cholesterol esters of blood plasma have been believed to be the palmitate and oleate. Without comment on the presence of palmitate, which seems well established, present work in this laboratory indicates that instead of cholesterol oleate the other ester is mainly linoleate, or at least the ester of a fatty acid containing more than one double bond. There can be no reflection on Hürthle's work, which was accurately done, but the elementary analysis on which he depended for his information was not sensitive enough to distinguish between a compound with one and one with two double bonds and he did not make use of iodine number determination to give information on this point. By fractionation of the lecithin-free blood lipoids with ethyl or methyl alcohol the least soluble and largest fraction is found to consist almost entirely of a compound which on analysis gives one molecule of cholesterol to one mole-

cule of a fatty acid with an iodine number varying from 120 to 170 and averaging about 145 (while for oleic acid the I.N. would be 90). The important fact is thus brought out that the highly unsaturated fatty acids found in blood plasma are carried to a considerable extent in combination with cholesterol. The significance of this fact is not altogether clear, for while it seems to connect cholesterol closely with the intermediary metabolism of the fatty acids, the cholesterol esters are, in contrast to lecithin, very stable compounds. It brings to mind the familiar experiments of Knoop<sup>24</sup> who found on feeding phenyl derivatives of the fatty acids that the fatty acid chains were reduced two carbons at a time back to within one or two carbon atoms of the ring. It may be that cholesterol forms such an unburnable 'handle' from which the fatty acids are burned.

The next step in Leathes' hypothesis is the breaking by oxidation of the long chains at the points of desaturation into shorter chains the length of which depends on the number of double bonds. Thus ordinary oleic acid would yield azelaic and pelargonic acids.



The constitution of the linoleic acids (two double bonds) is not well understood, but such a one as below with one double bond between the 6th and 7th and one between the 9th and 10th would yield caproic, malonic and azelaic acids.




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\* Cholesterol and lecithin are known to be opposite in their effects on hemolysis, lecithin producing hemolysis under the influence of cobra venom (see note above) while cholesterol protects against hemolysis by saponin<sup>25</sup> agaricine and tetanolysin<sup>26</sup>. The occurrence of cholesterol esters of the highly unsaturated acids in blood and the fact that these acids appear to be readily separable from lecithin suggests a possible origin of the cholesterol esters of the unsaturated acids. When set free from the lecithin by esterases in the blood which are known to attack lecithin, the acids are taken up by cholesterol, the combination serving two purposes—a neutralization of the hemolytic action of the unsaturated acids, and the use of cholesterol in the further metabolism of the fatty acids, possibly as an unburnable residue from which the fatty acids are consumed.



Fatty acids with two double bonds in other positions would yield different split products. Fatty acids with three double bonds are found in various organs — such a one as, for example:  $\text{CH}_3(\text{CH}_2)_3 \cdot \text{CH}=\text{CH} \cdot (\text{CH}_2)_3 \cdot \text{CH}=\text{CH} \cdot (\text{CH}_2)_2 \text{COOH}$  would yield valeric, glutaric (or butyric), succinic, or propionic acids. Arachidonic acid  $\text{C}_{20} \text{H}_{32} \text{O}_2$ , found by Hartley<sup>27</sup> in liver and by Levene<sup>17d</sup> in liver lecithin, having four double bonds might be expected to yield mainly acids with less than five carbon atoms. (The position of the double bonds is a matter of not inconsiderable importance since it was pointed out by Lewkowitsch<sup>28</sup> that if the double bond is near the carboxyl group iodine absorption takes place very imperfectly and its extent varies with the iodine solution used.)

How far is desaturation carried? Fatty acids with more than four double bonds are suspected but not actually found, for with the increase of double bonds the susceptibility to oxidation increases so greatly that the practical difficulties render preparation very difficult if not impossible. However, it can readily be seen from the above that if desaturation with subsequent breaking went far enough any other method of oxidation, as for example beta oxidation, would be unnecessary.

As noted above, desaturation as a preliminary stage in oxidation may be accepted as a fact. What evidence is there of the breaking of the chain into shorter fragments with separate oxidation as further required by the hypothesis? Before proceeding to a discussion of this phase of the subject it will be well to review briefly the present ideas regarding the method of final oxidation of the fatty acids. It is now pretty generally accepted that oxidation is brought about by removal of the carbon atoms two at a time with a preliminary oxidation of the  $\beta$  carbon atom which after the break becomes the terminal carboxyl group. The two-carbon fragment produced by this method of oxidation is ordinarily assumed to be acetic acid. It should be noted that while the fatty acids of longer chain than acetic acid were found by Schotten<sup>29</sup> to be completely burned, acetic acid in the same amounts was only 90% burned, although smaller amounts were completely utilized. It is also interesting to quote in this connection the work of Loeb<sup>30</sup> and Friedman<sup>31</sup> that acetates give rise to diacetic acid in perfused livers which are poor in glycogen. When much glycogen is present in the liver or when salts of pro-



pionic or valeric acids are present the change is entirely inhibited<sup>32</sup>. It is not necessary at this time to go into the details of evidence regarding the  $\beta$  oxidation of the fatty acids since this material is excellently reviewed by Dakin<sup>33</sup>. The outstanding facts are as follows: (a) the occurrence of the "acetone bodies" ( $\beta$  oxidized products) in the urine under certain conditions which may be summed up briefly as the lack of "available" carbohydrates in metabolism. These acetone bodies are now believed to originate mainly in the fatty acids and to represent their unburned residues. (b) The experiments of Knoop<sup>24</sup>, feeding phenyl derivatives of the fatty acids and studying the excreted products, gave strong indication that  $\beta$  oxidation is the prevailing method of oxidation in the animal body both for chains with odd numbered carbon atoms as well as for those with even numbered carbon atoms, for saturated as well as unsaturated acids. The objection that  $\beta$  oxidation is not the rule *in vitro* has been largely overcome by the experiments of Dakin<sup>34</sup> with hydrogen peroxide at about body temperature, by which he showed that  $\beta$  oxidation is the rule under these conditions, which closely parallel those in the living body.

Further evidence has been obtained by perfusion of surviving organs with various fatty acids (Embden<sup>35</sup>), and by excessive feeding with fatty acids (Blum<sup>36</sup>).  $\beta$  oxidation is thus shown to be very probable and in fact the only method of oxidation in the living body for which there is satisfactory evidence.

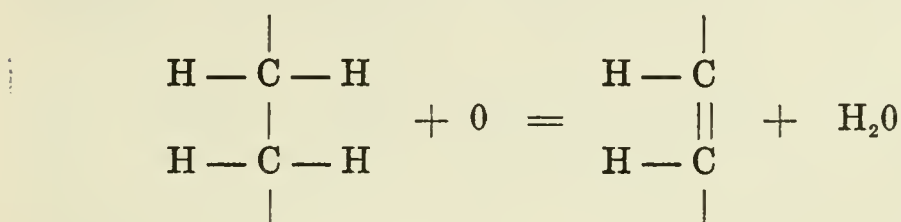
Incidentally it has been shown by these experiments that it is only the even numbered carbon atom fatty acids that yield acetone bodies. Odd numbered fatty acids do not, — in fact Ringer<sup>37</sup> has demonstrated that the odd numbered fatty acids yield glucose to the extent of three carbon atoms to each chain, the sequence of changes being probably propionic to pyruvic to lactic acids and thence to glucose,  $\alpha$  oxidation taking place when  $\beta$  oxidation is no longer possible, a probability given sound support by the recent work of Blum and Woring<sup>38</sup>.

Many years ago it was shown by Magnus-Levy<sup>39</sup> that in the completely diabetic organism each fatty acid chain yielded only 1 molecule of  $\beta$ -oxybutyric acid, (the maximum yield  $\beta$ -oxybutyric acid from 100 gm. of fat being 36 gm., i.e. 3 mols. from each molecule of fat). His results of course refer only to even numbered chains which are the only ones occurring in natural fats.

Work with shorter chain even numbered fatty acids as noted above confirmed his findings for the shorter chains and there is at present no evidence to show that an even numbered fatty acid chain of whatever length can yield more than one molecule of acetone bodies. The bearing of these facts on Leathes' hypothesis of the breaking of the long chain acids at the double bonds into shorter chains with individual oxidation will be clear from the following: The shorter chains formed by breaking the long chain would yield either odd or even numbers of carbon atoms. The even numbered ones would each produce one molecule of acetone bodies so that there would be as many molecules of acetone bodies as there were even numbered fragments formed. The odd numbered chains would yield glucose to the extent of three carbon atoms to each chain so that each pair of odd numbered fragments would produce one molecule of glucose. However, the evidence is contrary to the production of sugar from fat either in the natural diabetic or in experimentally produced diabetes. For example, Lusk has shown that fat feeding did not affect glycosuria either in phlorizinized dogs<sup>40</sup> or in diabetes<sup>41</sup>; also<sup>42</sup> that work which doubled fat catabolism did not alter the output of sugar in a phlorizinized dog. Some indirect evidence from respiration experiments is available, for example, the occasional R. Q. below 0.7 in diabetes and in depancreatized dogs, which is interpreted by some writers as evidence of change of fat to sugar, but is probably better explained by the desaturation of the fatty acid (see below). In the normal the most significant recent work on this point is that of Krogh and Lindhard<sup>1</sup>, who as the result of studies of the respiratory quotient at various levels come to the conclusion that at quotients above 0.9 carbohydrate is transformed into fat and at quotients below 0.8 fat is transformed into carbohydrate. Without comment on the transformation of carbohydrate to fat which is known to take place readily, their conclusions with regard to the transformation of fat to carbohydrate may without injury to their conceptions be restated as follows. The abnormally low R. Q. is due to transformation of the fatty acid into substances containing *more oxygen* or *less hydrogen* than the fatty acid (but not necessarily carbohydrate). Such substances would be the desaturated or hydroxy fatty acids, and so their findings may be brought into agreement with the desaturation hypothesis, since there would in these cases be utilization of oxygen without a cor-



responding output of  $\text{CO}_2$ . It is known that it is at just such times (low carbohydrate, high fat combustion) that there is a flow of fat to the liver as the result of its low glycogen content, and the adherents of the carbohydrate from fat hypothesis have accepted this along with the reduced R. Q. as evidence for their theory<sup>43</sup>. It is interesting to note that Geelmuyden<sup>43</sup>, who has been one of the most insistent supporters of the fat to sugar hypothesis, accepts desaturation as the first stage in the transformation and gives the following diagram to illustrate what he believes to be the first stage in the transformation of fatty acid to sugar.



The use of oxygen in this way and also possibly in the formation of hydroxy or ketone acids would explain the lowered quotient if the unsaturated acids were stored, and Leathes has shown that they are stored to some extent in the liver. The apparent formation of fat from carbohydrate in these experiments can thus be shown to be stages in the utilization of the fats, and the apparent contradiction to Lusk's evidence regarding the transformation of fat to sugar is removed.

In summing up the evidence regarding the breaking of the fatty acids at the double bonds we are led to an absurdity. For if more than one even-numbered fragment were formed, then more than one molecule of acetone bodies per long chain fatty acid would be produced, which is not the case; while if odd numbered fragments were formed sugar would be produced from fat, which also appears not to be true. The conclusion is that the evidence is against the breaking of the long chains, which therefore must be reduced entirely by  $\beta$  oxidation. There are two possibilities of escape from this conclusion which should be considered: first, that sugar and acetone bodies may be produced in such amounts that they mutually consume each other, which is unlikely although not beyond the bounds of possibility; and second, that the dicarboxy acids which are formed by break-



ing the chain have a different path in metabolism from the monocarboxy acids. Evidence on the latter point is available from the work of Ringer<sup>37b</sup>, who found that malic and succinic acids administered to phlorizinized animals yielded large amounts of glucose, which, from his work on the odd numbered carbon fatty acids, he believed happened by loss of CO<sub>2</sub> resulting in a three carbon acid. Some light is also thrown on the subject by the behavior of dicarboxy amino acids. Ringer and Lusk<sup>44</sup> found that aspartic acid yielded much extra glucose, by loss of CO<sub>2</sub> and oxidation at the  $\alpha$  amino group. Similarly, glutamic acid went to glucose via succinic acid with loss of one carboxyl. In the case of these two amino acids which differ by one carbon atom but yet both yield glucose, the terminal carbon atom behaves differently, in one case disappearing, in the other persisting. Dakin<sup>45</sup> showed that arginine, ornithine and proline yield glucose, presumably as Ringer suggests via succinic acid, while lysine, which would go to glutaric acid, apparently does not yield glucose. The work quoted shows as far as it goes that the dicarboxylic acids form glucose and in that way are not different in their behavior from the odd numbered monocarboxylic acids. Of the other dicarboxylic acids not much is known. As regards glutaric acid Ringer states that it yields neither glucose nor  $\beta$ -oxybutyric acid. Leathes' own belief regarding the dicarboxylic acids is that they lose CO<sub>2</sub> and thus become monocarboxylic acids, after which they are oxidized by  $\beta$  oxidization.

Taken altogether the available evidence gives no support to the assumption that the long chain fatty acids are broken into parts for oxidation but rather that the chain remains intact to the end, the only changes being the introduction of an unknown number of double bonds which of course would render  $\beta$  oxidation easier.

A further possible change is the addition of hydroxyl groups at the double bonds. Hydroxy acids are found in the brain<sup>46</sup> where they constitute about 25 per cent of the solid acids. They have not been described as occurring elsewhere in animals, but as they are formed readily from the unsaturated acids and occur in plants, it is probable that they would be found if looked for. The fact that they are relatively soluble in water offers another possibility in the consideration of the soluble forms of the fatty acids. Dakin's review of this phase of the subject leads him to

the belief that the unsaturated fatty acids may take up water to form optically active, saturated hydroxy acids which then undergo further oxidation<sup>33</sup>. The conditions respecting the fatty acid compounds of the brain is worthy of comment. The energy exchange of brain and nerve tissue is relatively minute but the energy producing material must be of such a kind as to be instantly available. The oxygen supply is known to be very generous. The fatty acids found in brain tissue are among the most unsaturated found anywhere, lecithin and related phospholipoids are present in large amounts, hydroxy fatty acids are found in relatively large proportion and compounds of fatty acids with a sugar are found — a combination which does not occur elsewhere.

*Fatty Acid Synthesis.* — The naturally occurring fatty acids are all straight-chain compounds of even numbered carbon atoms and containing mainly sixteen or eighteen carbons, facts which point to a two carbon atom origin. Animal fat undoubtedly originates to a considerable extent from carbohydrate and the prevailing hypothesis regarding the transformation is that the carbohydrate is broken down to the two carbon stage, presumably to acetic aldehyde, and then by successive aldol condensations is recombined to form the various members of the fatty acid series. Shaffer<sup>47</sup> believes that this hypothesis is probably wrong, since at the first step, the aldehyde of  $\beta$ -oxybutyric acid, a ketogenic substance, is produced, which would make the carbohydrates ketogenic. But of course a ketogenic substance has to be formed at some time if fats are formed. The formation of diacetic acid from acetic acid in glycogen-free livers noted above should be considered in this connection.

*Abnormalities in Fat Metabolism.* — The recognized abnormalities of fat metabolism are few in number and the available information regarding them can be stated in a few words.

*Cholesterol.* — Cholesterol is practically insoluble in water and its presence in blood even in tenths of one per cent. forms a supersaturated solution which might be expected to deposit cholesterol when conditions were right. Actually deposition does not take place at normal and rarely at higher concentrations. Experimental hypercholesteremia in rabbits produces deposits of cholesterol, mainly in the form of esters; at various point, particularly in the walls of the arteries, and is believed by some to be a factor in the production of arteriosclerosis. The deposition



of cholesterol from the concentrated bile in the gall bladder in the form of gallstones is of course well known and some<sup>22</sup> have concluded that there is a causal relation between cholesterol and certain blood diseases such as the various anemias has been suggested but the evidence is not sufficient to warrant definite conclusions.

*Obesity.* — There is a prevailing popular belief in a certain mystery regarding obesity — that some people become fat on a diet that barely supports others. This belief has led to many experiments to discover if there is any essential metabolic difference between individuals, but the results have been mainly negative. The relation between surface area and energy requirement in the resting state holds for all individuals<sup>48</sup> and each piece of work requires (within narrow limits) a definite expenditure of energy which can be calculated and which applies to practically all individuals in the same way. Laying on fat means in general just one thing — that the energy intake exceeds the energy outgo — and can be satisfactorily treated on that basis. There are a few exceptions to this rule, such as adiposity of endocrine origin.

In addition to the disadvantages of carrying around extra weight in the form of fat there are certain dangers. One of these — the tendency of fat people to diabetes — may be either cause or effect, but it well known that diabetes in a great many cases, perhaps the majority, is preceded by obesity. The laying on of fat in beginning diabetes is perhaps to be explained as follows: There are two paths open to the carbohydrate of the food, combustion and storage, mainly in the form of fat. When the path to combustion is partly blocked through the lack of insulin, the first efforts of the organism is to save the carbohydrate by converting it into fat. Later with the increasing lack of insulin the difficulty of burning sugar increases, it accumulates in the blood and then begins to waste in the urine. Finally the wastage becomes so great that the food cannot supply the energy requirement and the accumulated fat melts away, producing the emaciation characteristic of the advanced diabetic. The tendency of even the severe diabetic to form and store fat is seen from the fact that after the ingestion of levulose the R. Q. often is exceptionally high<sup>49</sup> and also from the recently reported work of Richardson



and Mason<sup>50</sup> that the diabetic tends to burn more protein, more carbohydrate and less fat than he receives.

*Acidosis.* — The acidosis most frequently present in the animal body is that due to incomplete combustion of the fatty acid chain resulting in the production of the acetone bodies — diacetic and  $\beta$ -oxybutyric acids — which are relatively strong acids. As such they must be got rid of in the same way as the other strong acids produced in metabolism, i.e. by combination with bases and excretion as salts, with the result that in extreme cases the organism is robbed of the fixed bases which are necessary for respiration. There is no doubt that as the supply of available base becomes small the unneutralized acid in the cells may reach a dangerous concentration and the cell be permanently injured, which may explain the failure of alkali therapy in severe acidosis and the general downward progress of the whole metabolism when even slight acidosis is allowed to persist. Whatever may be said regarding the practice of allowing a moderate degree of glycosuria or a moderately high blood sugar with the idea that the greater concentration of sugar in the blood will produce by mass action a greater utilization — a conception which is in agreement with our present knowledge of such balanced reactions — there can be no question that the accumulation of acetone bodies even in small amounts cannot but be harmful even though more is consumed.

Why the oxidation of the long fatty acid chain should stop at the four carbon stage, and that when the initial stage ( $\beta$  oxidation) in the next step of oxidation has already taken place, is an interesting subject for speculation. It is generally believed that the short chain fatty acids are more easily oxidized than the long ones — as witness the belief in the breaking of the long chains. Also diacetic acid is a very active substance and so should be more easily disposed of than butyric acid. And yet it is just the stage that cannot be passed without the simultaneous combustion of carbohydrate. A possible reason for the break in the series of oxidations at this point is the increasing acidity of the fatty acids as the chain shortens. The long-chain fatty acids are very weak acids. With the shortening of the chain the residues become increasingly stronger acids until at the four carbon stage the acid is of significant strength and is still stronger by virtue of the oxygen in the chain. Another factor which may be of

importance is the increasing solubility of the residues. The long chain acids are insoluble in water, while butyric acid is relatively soluble and diacetic and  $\beta$ -oxybutyric acids still more so. It is possible that the increasing solubility and acidity reaches a stage at this point where conjugation with some part of the glucose molecule is necessary to overcome the injurious effect of these properties.

The relation of carbohydrates to the combustion of the fatty acids is an interesting problem and much work has been done on it by Shaffer, Woodyatt, Hubbard and others. It has been, however, the subject of a recent lecture by Dr. Shaffer<sup>47</sup> before this Society and so need not be detailed. The relation appears to be quantitative, one molecule of glucose being effective for the oxidation of two molecules of fatty acid. Under bodily conditions double that amount of glucose is necessary for complete avoidance of acetone body production due, as Shaffer suggests, to difficulties in distribution. His *in vitro* experiments indicate that glucose to be effective must be in process of oxidation. Having in mind the ready reactivity of aceto-acetic ester or its sodium salt, Shaffer has experimented with various aldehydes in the hope of getting some light on the glucose derivative which combines with diacetic acid to bring about its destruction. His experiments led him to no definite conclusion other than that glucose itself is not the effective substance.

Of interest in this connection is the recent announcement of Intarvin. Following up the early work of Ringer, who found that the odd numbered carbon fatty acids did not produce acetone bodies but instead sugar, Doctors Kahn and McKee of New York have synthesized a fat in which the fatty acids are all long chain (C 17) odd numbered fatty acids, with the idea that thereby the nutriment of fat could be provided without danger from acidosis. The subject is apparently still in the early experimental stage, for very little information is available concerning it. There seems to be no doubt that the new fats are absorbed and that no acetone bodies are produced — the acetone body excretion drops at once when feeding with this fat begins and as promptly rises when ordinary fat is substituted — but that all may not be clear sailing is indicated by the fact reported from one laboratory that the organic acidity as determined by Van Slyke's method is scarcely affected by the change and that therefore there may be



a replacement of one organic acid for another. The experiment is an important one and will be followed by all of us with interest.

*Lipemia.* — The blood plasma in the post-absorptive condition is generally clear, or at least free from suspended fat particles. Normally a meal of fat is absorbed into the blood stream and has disappeared again from it in the course of twelve to fourteen hours. A milkiess that persists longer than 24 hours after a meal is probably to be regarded as abnormal. Such a milkiess of the plasma may appear in fasting, due to very rapid discharge from the fat stores. It may occur as the result of extensive hemorrhage in some animals, as for example the rabbit, where the source is again largely the fat of the stores and probably particularly in this case the bone marrow, although the lipemia is greater when the animal is given fatty food<sup>14</sup>. It is relatively frequent in severe diabetes, in which the source is undoubtedly the fat of the food. But no matter what the source, the development of the lipemia and its final form is approximately the same. The lipoid constituents increase in the following order, first the fat, then the lecithin and third the cholesterol. As the lipemia persists the cholesterol continues to increase, while the lecithin after reaching a certain value remains stationary. There is a tendency toward a balance between cholesterol and lecithin such as exists in fasting plasma, but in long lasting lipemia the greater increase of cholesterol results in a disturbance of the balance.

The occurrence of lipemia may in most cases be explained as a disturbance in the balance between inflow and outflow of fat. In diabetic lipemia the disturbance appears to be in the outflow, because a high grade lipemia lasting two or three weeks may sometimes be produced by a single fat feeding<sup>13</sup>, and in other cases a lipemia of unknown origin may persist for an equally long time on a fat-free or low fat diet. In fasting and hemorrhagic lipemia the disturbance is caused by an abnormally large inflow, since the disappearance of the lipemia is rapid. The mechanism of diabetic lipemia has been worked out by Allen<sup>51</sup> in dogs made experimentally diabetic by removal of a large proportion of the pancreas and suitable feeding. The conditions under which lipemia occurred in his animals were two in number, first and apparently most important was the existence of active, severe symptoms in the form of glycosuria and hyperglycemia, and second a sufficient supply of fat in the diet. Mild cases even with



high glycosuria and severe cases in which the glycosuria has been abolished by diet never exhibit any extreme grade of lipemia no matter how high the fat intake. The lipemia then represents not a primary disturbance in fat metabolism but is a secondary phenomenon due to the breakdown in carbohydrate metabolism as the result of endocrine disturbance. Wide variations in susceptibility are found in both animals and patients.

Allen's conclusions are compatible with those of Joslin<sup>52</sup> obtained by a study of blood lipoids in a large number of diabetics. Joslin's findings are that diabetics with the lowest metabolic level and most below standard body weight have the highest blood fat. Apparently any treatment which will improve the metabolic condition of the diabetic will abolish the lipemia. Most remarkable is the fact that feeding of fat to the severe diabetic with lipemia will often cause a disappearance of the lipemia<sup>53</sup> presumably through an improvement in his condition, the fat of the food sparing body protein<sup>54</sup>.

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## THRICE-COOKED VEGETABLES FOR DIABETICS

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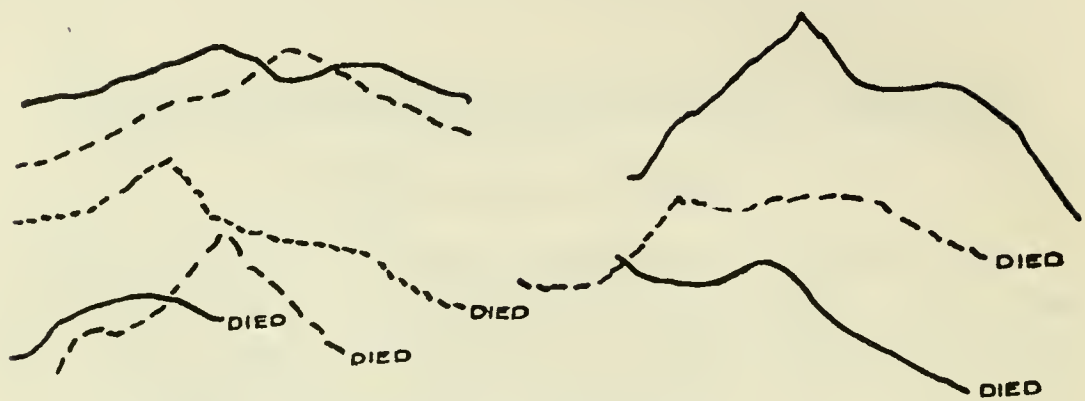
Thrice-cooked vegetables are frequently prescribed for diabetics both during the starvation period at the beginning of treatment and also thruout the treatment of severe cases. The liberal use of thrice-cooked vegetables is recommended when the total calories are very restricted, since it brings the quantity of food somewhere near normal without adding materially to the fuel value of the diet. It is sometimes very difficult to satisfy the patient and prevent sensations of hunger when the daily dietary is below 1000 calories without resorting to thrice-cooked vegetables.

Croll recently made a valuable contribution to the study of diabetic foods by publishing<sup>1</sup> the determination of the protein, carbohydrate, and fat in a large number of thrice-cooked vegetables. They are calculated as nothing by most physicians and dietitians. Croll's results show that the 5% class which are sometimes calculated as a class as averaging 3% carbohydrate, are reduced to approximately one-half per cent carbohydrate. The protein and fat are also somewhat reduced. Not much interest has been shown in the mineral or vitamine content of the diabetic diet, as it has seemed to have no direct relation to the disease. The patient who uses the 5% vegetables raw or once cooked undoubtedly has an abundant supply.

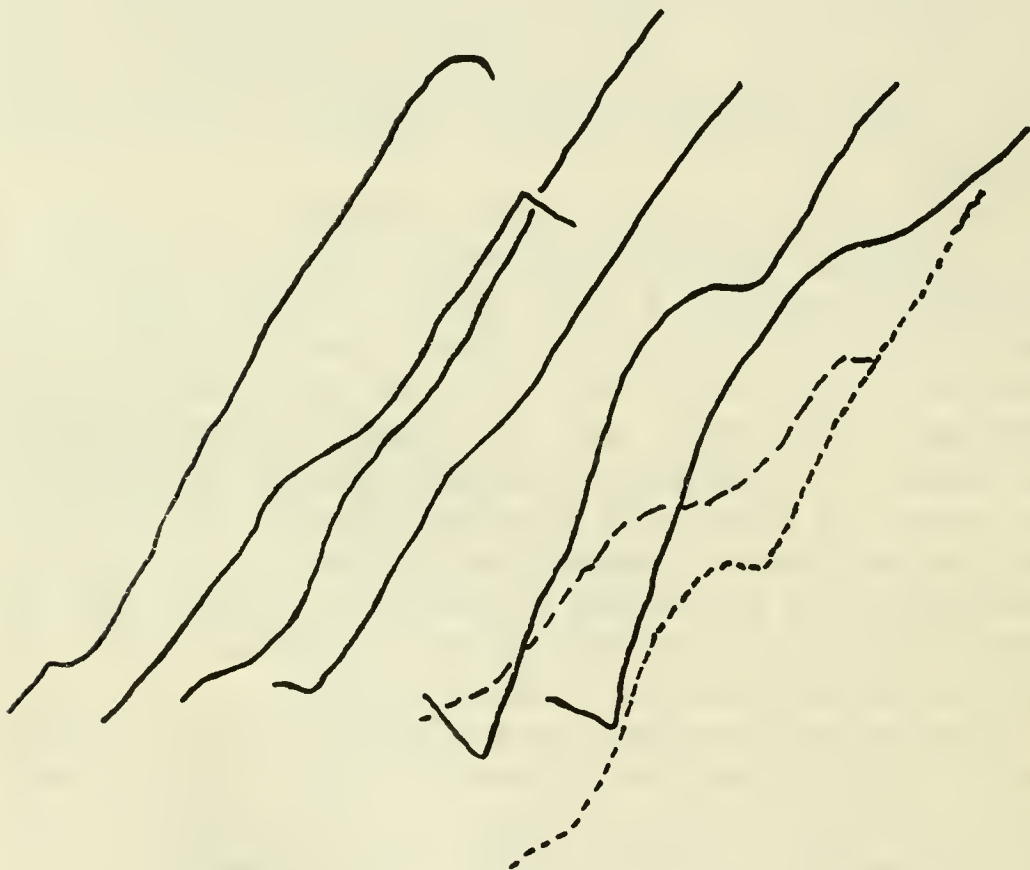
Since we had determined in our laboratories the minimum amount of raw spinach which would support normal growth in the rat and had compared with it the growth produced with the same quantities of various preparations of spinach, we were interested in adding to our data the growth produced by the same quantity of thrice-cooked spinach, altho there are already recorded experiments which indicate there is a large proportion of vitamine extracted in cooking water<sup>2</sup>. It would therefore be natural to infer that the thrice-cooked vegetable would be much poorer in vitamine than the vegetable cooked and drained in the usual manner.

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THRICE COOKED SPINACH



RAW FRESH SPINACH

## EXPERIMENT

Young rats 40-55 days old were used, and the usual care was employed to keep them clean and prevent their having access to their feces. The basal diet was the same as that used previously by us: 15% purified casein, 10% crisco, 72% corn starch and 3% salt mixture (Gulick's formula). The basal diet

was furnished in unrestricted quantity. The vegetable, which was intended as the only source of vitamine, was given daily in weighed amounts. Eight rats were given 4 gm. of raw spinach, and eight were given 4 gm. of thrice-cooked spinach. About 75 gm. of vegetable was used to 1 liter of water, and it was cooked 10 minutes each of three times.

The raw spinach furnished satisfactory growth, similar to that obtained in two previous series; but no growth was obtained with the thrice-cooked spinach. On the twenty-fifth day, the first of the eight died; the second died on the thirty-first day. The fortieth day another died and the internal organs were eaten by those in the cage with it. We were interested to see whether the survivors would gain in weight following this, but no gain was observed. On the forty-seventh and forty-eighth days two more died, one being eaten by the survivors. The experiment was then discontinued. Some experiments with cabbage indicated that these results are not confined to one vegetable.

### DISCUSSION.

The thrice-cooked vegetable could not be given in increased doses, as the rats would not take more of it. On some days they did not consume all of their 4 gm. dose. The reason for discontinuing the experiments was the fact that spinach could be obtained no longer. From this experiment it would seem that if thrice-cooked vegetables are not totally devoid of vitamine, the amount retained is insignificant, and they cannot be counted upon as a source of vitamine in the diabetic dietary. No attempt was made to determine which of the vitamins were lacking.

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## EXPERIMENTAL STUDIES IN DIABETES.

### SERIES V. — ACIDOSIS.

#### 7. — THE INFLUENCE OF RENAL LIGATIONS AND INJURIES ON KETOSIS.

By FREDERICK M. ALLEN.

In the classical literature, the reduction or suppression of phlorizin glycosuria by every form of spontaneous or experimental nephritis is familiar, and sometimes the disappearance of an accompanying ketonuria has also been noted. In a former instance in this series<sup>1</sup> spontaneous nephritis in a dog seemed to confer an unusual immunity against ketosis. Again,<sup>2</sup> thrombosis of the vena cava involving the renal veins seemed to have a similar effect. Experiments were therefore undertaken to follow up these observations. With the variations introduced for control purposes, they may be classified in the following groups: (1) Partial ligation of vena cava; (2) partial ligation of renal veins; (3) partial ligation of renal arteries; (4) partial ligation of aorta; (5) ligation of ureters; (6) partial or total nephrectomy; (7) injections of acetone bodies; (8) partial or complete pancreatectomy and partial ligation of renal veins.

#### METHODS.

Little need be mentioned except the operation for partial ligation of vessels. The ligation of the renal veins must be tight enough to cause marked swelling and prolonged stasis in the kidneys. If the ligatures are placed too tightly acute death results, and if too loosely the desired effects are slight or absent. After various failures, the method finally adopted was to place a wire of suitable size alongside the renal vein and tightly ligate both wire and vein in a single loop of silk. Withdrawal of the wire then left a small but definite lumen open in the vein.

Obstruction of the vena cava or aorta involved a maximum of disturb-

ance in other organs, while the opposite extreme was represented by ligatures of the renal vessels close against the kidneys, affecting the kidneys alone. In order further to exclude an assumption that the left adrenal, in particular, might be affected by interference with the renal circulation on that side, comparisons were made by ligating the renal veins close to the kidneys in some animals and close to the vena cava in others, with identical results. In the majority of instances conditions were made more rigid by stripping the kidneys of their fatty capsule or other connections that might carry collateral circulation.

The great flaw in the chemical work lay in the inability to make quantitative analyses of the acetone bodies under the existing circumstances. The necessity of judging the presence, absence, or degree of ketosis merely by the qualitative nitroprusside reactions in urine and plasma and the CO<sub>2</sub> capacity of the plasma left unfortunate openings for criticism. For the crude purpose required by these experiments, namely distinguishing between heavy ketosis on the one hand and faint or absent ketosis on the other, these tests are in fact probably adequate. Nevertheless it seemed inadvisable to publish this work until there was opportunity to repeat certain typical experiments and demonstrate the changes in the acetone bodies quantitatively under these conditions, as has been done in the ensuing paper (No. 8) of this series.

### 1. PARTIAL LIGATION OF VENA CAVA.

*Dog G7 - 20* was a male mongrel, mixed Dalmation and Newfoundland, aged about 4 years and weighing 19 kg. in fair nutritive condition.

TABLE 1.

*Dog G7 - 20.*

Date 1918	URINE					BLOOD PLASMA		REMARKS
	Vol. cc.	Sugar gm.	Total N gm.	D/N ratio	Nitro- prusside	Sugar mg. %	CO <sub>2</sub> Cap. Vol. %	
June 24	1330	45.9	3.20	—	neg.	—	—	1 gm. phlorizin.
„ 25	1280	45.9	11.44	4.00	faint	—	—	
„ 26	500	10.3	5.05	2.40	neg.	—	—	
„ 27	1170	24.0	10.08	2.22	faint	75	51.9	1 gm. phlorizin.
„ 28	1070	19.9	8.80	2.26	neg.	—	—	
„ 29	1125	15.6	—	—	„	—	—	
„ 30	890	8.8	9.81	0.89	„	90	44.3	Fed 300 gm. lung, 100 gm. suet.
Aug. 16	900	8.8	4.68	2.84	„	102	68.1	Blood: Urea=25.5 mg. %
„ 17	840	13.1	12.06	1.09	„	—	—	1 gm. phlorizin.
„ 18	500	7.3	—	—	„	—	—	1 gm. phlorizin.
„ 19	600	9.9	7.38	1.34	„	88	54.2	



June 18, 1918, a heavy silk ligature was placed about the vena cava just cephalad from the renal veins, causing as much constriction as seemed compatible with survival of the animal. The cava was bulging and tense below the ligature, and the kidneys were perceptibly congested. The dog was well after the operation except for apparent weakness of the hind legs and poor appetite. After a few days the behavior seemed entirely normal, but slight albuminuria persisted.

June 24 to 30, the dog fasted and received two subcutaneous injections of phlorizin, as shown in table 1. Ketonuria was barely perceptible by qualitative tests and was not measurable quantitatively. The feeding of 300 gm. beef lung and 100 gm. suet after the close of the experiment on June 30 also resulted in no ketonuria in the ensuing 24 hours. The dog became weak, but had no clinical symptoms of acidosis during the fast.

Mixed diet was then eaten in large quantities, and strength and weight were rapidly recovered. Aug. 16 to 19, another fasting period was imposed, with two injections of phlorizin. The dog became so dangerously weak that the fast had to be terminated on the 19th, but there was no trace of acidosis.

Considerable oatmeal mixture was eaten on Aug. 20, but weakness increased and death occurred on Aug. 21. Autopsy was negative grossly and microscopically except for chronic congestion in the kidneys and all other organs below the venous ligature. The death seemed not explainable anatomically. The only analyses for nitrogen retention were those at the end of the first fast, when the blood urea was normal. The weakness at the close of the second fast was similar but more pronounced, and there was no suppression of urine such as might suggest uremia. By comparison with the long survival of other dogs with venous stasis of the kidneys alone, death was probably due here to other injurious effects of the caval ligation, and was not from the kidneys alone.

#### REMARKS.

The partial occlusion of the inferior vena cava by ligature seemed to reduce the susceptibility to acidosis with fasting and phlorizin, as observed in the case of accidental thrombosis. Glycosuria and the D:N ratio were also low especially in the second fast.

*Dog G7-57* was a mongrel Collie pup, aged about 2 months and weighing 2.4 kg. in medium nutritive condition. July 2, 1918, a silk ligature was placed about the vena cava just cephalad from the renal veins, producing the utmost constriction that seemed safe. The animal recovered quickly and drank some milk in the afternoon of the same day.

Fasting was begun on July 3. A faint nitroprusside reaction appeared in the urine on July 5, and increased to moderate on July 6, but became negative on July 7 and 8. Slight albumin and small numbers of red corpuscles were demonstrable in the urine throughout. Clinical symptoms of

acidosis remained absent. The pup was thin and moderately weak when the fast was ended on July 9, but the entire behavior seemed to contrast with that of normal puppies of this age.

Notwithstanding the eating of mixed diet, weakness continued to increase and became dangerous on July 13. The urine was normal in quantity and showed the usual characters. On July 13 the abdomen was opened and the ligature about the cava removed, with a view to saving the animal's life and also permitting comparison with a subsequent fasting period without the venous obstruction. There was no benefit and death occurred on July 14.

The emaciated cadaver weighed only 1500 gm., and the mere cachexia was a sufficient cause of death. The only striking gross changes were in the kidneys. The left one was enlarged and weighed 11.8 gm. The right one was perhaps atrophic, weighing only 6.8 gm. Its consistency seemed slightly increased. Otherwise both kidneys had an approximately normal appearance, and their capsules stripped readily. Only the kidneys were examined microscopically. Both showed the usual results of venous stasis, but the collapse of tubules and interstitial changes were much more marked in the right one. A few casts were visible in this kidney.

#### REMARKS.

The ligature of the inferior vena cava so as to congest the kidneys and lower viscera seemed to render this puppy insusceptible not only to ketosis in a chemical sense but also to the accompanying symptoms. The behavior in both these respects appears as an exception to the rule of fasting ketosis which is invariable as far as observed in normal puppies of this age.

#### 2. PARTIAL LIGATION OF RENAL VEINS.

As the quick reduction or abolition of ketosis by this operation seems to be a constant phenomenon, and further examples are found among the subsequent groups, only one typical protocol is given out of a series available under this head. In addition are given two control observations, and a ligation experiment in a dog with a reversed Eck fistula.

*Dog G7-67* was a male Boston terrier mongrel, aged 5 years and in good condition at a weight of 12 kg. Fasting and phlorizin were begun on July 8, as shown in table 2.

July 13, under the usual ether anesthesia, silk ligatures were placed about both renal veins as tightly as seemed compatible with life. The D:N ratio in the urine passed after the operation (3.22:1) was higher than at any previous time (perhaps because of the anesthesia), but the acetone reactions previously present in blood and urine cleared up practically im-

TABLE 2.  
*Dog G7 - 67.*

Date 1918	URINE					BLOOD PLASMA			REMARKS
	Vol. cc.	Sugar gm.	Total N gm.	D/N ratio	Nitro- prusside	Sugar mg. %	Nitro- prusside	CO <sub>2</sub> Cap. Vol. %	
July 8	290	8.55	3.69	2.31	neg.	—	—	—	Not fed. 1 gm. phlorizin
" 9	320	13.56	6.08	2.23	faint	—	—	—	" " 1 gm. phlorizin
" 10	300	7.68	5.94	1.29	mod.	—	—	—	" " 1 gm. phlorizin
" 11	260	10.20	5.61	1.82	"	—	—	—	" " 1 gm. phlorizin
" 12	280	11.20	6.09	1.93	heavy	—	—	—	" " before operation
" 13	190	8.22	—	—	"	89	slight	56.7	" " after "
" 14	150	4.12	1.28	3.22	v.faint	—	—	—	" "
" 14	385	5.16	—	—	neg.	147	neg.	52.8	" "
" 15	835	8.82	4.41	2.00	"	—	—	—	" "
" 16	870	11.52	10.35	1.11	"	128	neg.	47.1	50 gm. meat. Urea 181.9 mg. %
" 17	870	faint	5.04	—	"	—	—	—	Refuses food.

mediately. Up to July 16, the glycosuria and D:N ratios were only slightly lower than before, but nitroprusside tests remained negative in urine and blood plasma, and the plasma bicarbonate fell no lower than 47.1 volumes per cent.

The dog refused food when it was offered on July 17, grew weaker, and died on July 20. Since the operation, the urine had contained moderate amounts of albumin, casts and red corpuscles, and the high blood urea of July 16 suggested uremia as the cause of death. The autopsy revealed no gross cause of death, and microscopic examinations were negative except for the usual congestive pictures in both kidneys.

#### REMARKS.

The clearing up of acetone reactions in both blood and urine seemed too striking to be accounted for by the slight fall in glycosuria and D:N ratios. This clearing up also seemed to begin immediately after the operation, when the D:N ratio was particularly high. As far as we have ever seen, dogs with any marked acetone-body retention always show positive nitroprusside reactions in the plasma, and the negative plasma reactions here seemed to exclude mere retention of acetone due to renal impermeability.

*Dog G7 - 89*, a female bulldog aged 4 years and in good condition at a weight of 17.5 kg., was started fasting on July 30, 1918. Subcutaneous injections of 1 gm. phlorizin were given on July 30 and 31 and Aug. 2. By



Date 1918	URINE				BLOOD PLASMA			REMARKS
	Vol. cc.	Sugar gm.	Total N gm.	Nitro- prusside	Sugar mg. %	Nitro- prusside	CO <sub>2</sub> Cap. Vol. %	
July 30	140	3.05	—	neg.	—	—	—	Not fed. $\frac{1}{2}$ gm. phlorizin.
" 31	90	3.71	—	slight	—	—	—	" " Urine before operation.
Aug. 1	40	2.46	3.41	heavy	133	mod.	34.6	" " after "
" 2	37	1130	—	slight	—	—	—	" " "
" 3	107	4122	2.09	faint	156	mod.	30.8	" " "
" 3	—	—	—	—	132	neg.	18.8	Blood Urea 63.3 mg. %

On Aug. 1, after heavy ketosis had developed, both renal veins were tightly ligated, with a view to stopping all circulation. The animal gradually became weak, but continued to pass urine. While the blood sample was being taken from the jugular on Aug. 3, a slight convulsion occurred and ended in death.

At autopsy the right kidney was found two or three times the normal size, and completely black. The left kidney was not very seriously congested, and the reason was found in a small supernumerary vein issuing from the hilum, which had not been ligated.

## REMARKS.

Slight hyperglycemia was present. Ketosis did not clear up immediately after the operation. The result may be due partly to general prostration and not entirely to renal deficiency, since any state of profound weakness and collapse may be attended by a clearing up of acetone in experimental animals.

TABLE 5.

*Dog F6 - 30.*

Date 1918	URINE					REMARKS
	Vol. cc.	Sugar gm.	Total N gm.	D/N ratio	Nitro- prusside	
June 17	730	25.2	5.8	4.82	neg.	Not fed. 1 gm. phlorizi
" 18	1100	59.5	13.7	4.92	"	" " " "
" 19	485	43.5	12.6	3.65	slight	" " " "
" 20	975	31.7	15.6	2.62	heavy	" " 1 gm. phlorizin.
" 21	700	47.0	—	—	"	" " " "
" 22	770	44.4	13.4	3.54	"	" " 1 gm. phlorizin.
July 16	1125	6.5	—	—	neg.	Not fed. 1 gm. phlorizin.
" 17	1500	7.1	9.0	0.79	"	" " " "
" 18	1120	15.8	6.2	2.55	"	" " 1 gm. phlorizin.
" 19	1285	10.5	8.9	1.18	"	" " " "
" 20	1310	4.8	10.8	0.48	"	" " 1 gm. phlorizin.
" 21	1400	10.6	10.8	1.00	"	" " " "
" 22	960	10.8	—	—	"	" " 1 gm. phlorizin.
Aug. 16	550	12.7	7.0	1.80	neg.	Not fed. 1 gm. phlorizin.
" 17	960	15.3	10.0	1.53	"	" " " "
" 18	700	14.4	10.0	1.44	faint	" " " "
" 19	600	12.6	9.0	1.40	slight	" " 1 gm. phlorizin.
" 20	800	13.1	9.8	1.34	"	" " " "
" 21	500	9.6	7.0	1.37	faint	" " " "

*Dog F6 - 30* was used for a reversed Eck fistula experiment, as described in the preceding paper.\* June 15, 1918, fasting was begun. Injections of phlorizin were given on June 17, 18 and 20, as shown in table 5. Heavy nitroprusside reactions persisted through June 22, when the experiment was discontinued.

July 13, both renal veins were ligated as tightly as possible without complete occlusion. The dog at first vomited and passed scanty bloody urine, but gradually recovered normal behavior.

July 16, the urine still contained considerable albumin and red cells. Fasting was begun, and 1 gm. phlorizin injected subcutaneously. Additional phlorizin was given on July 18 and 20, as shown in table 5. No ketosis was shown by the tests of urine or blood. Feeding was resumed July 23.

By Aug. 14 only a faint trace of albuminuria persisted. On this day fasting was begun. 1 gm. phlorizin was injected subcutaneously on Aug. 16, 17 and 19, as shown in table 5. Very slight ketosis resulted.

#### REMARKS.

If any increased tendency to ketosis existed by reason of the reversed Eck fistula, it was evidently overcome by the renal vein ligation. The suppression of ketosis was less complete in the final experiment (in August), perhaps because the kidneys had established a better collateral circulation with time; but the dog later came to autopsy, and both the persistence of the reversed Eck fistula and the chronic renal stasis were confirmed. As usual, the D:N ratios were lower after the renal vein ligation than before, and the question is whether this smaller glycosuria was the only cause of the suppression of ketosis.

### 3. PARTIAL LIGATION OF RENAL ARTERIES.

*Dog G7 - 75* was a yellow mongrel aged 4 years, in good condition at a weight of 12.6 kg. Fasting was begun July 16, 1918, and doses of 1 gm. phlorizin in oil were injected subcutaneously on each day.

July 20, both renal arteries were ligated with silk as tightly as seemed possible without stopping the circulation completely. The dog died on the following day, with no symptoms except weakness. Before operation, the urine contained 13 to 16 gm. of sugar daily and gave heavy acetone reactions. From operation to death, the dog passed 225 cc. of urine which was free from both sugar and acetone. Autopsy showed no cause of death. There was no necrosis in the kidneys, and the renal arteries seemed to be still slightly patent.



## REMARKS.

Interference with the renal function by partial ligation of both arteries, perhaps with an additional influence of shock, here seemed able to suppress both phlorizin glycosuria and acetonuria.

*Dog G7 - 83*, a male mongrel aged 6 years and weighing 12 kg. in good condition, on June 22, 1918 started fasting and received 1 gm. phlorizin in the usual oil suspension subcutaneously. Ketonuria appeared on July 24, and another phlorizin injection was given on this day, as shown in table 6.

TABLE 6..

*Dog G7 - 83.*

Date 1918	URINE				BLOOD PLASMA				REMARKS
	Vol. cc.	Sugar gm.	Total N gm.	Nitro- prusside	Sugar mg. %	CO <sub>2</sub> Cap. Vol. %	Nitro- prusside	CO <sub>2</sub> Cap. Vol. %	
July 24	1780	28.8	8.20	slight	—	—	—	—	Not fed. 1 gm. phlorizin subcut.
„ 25	340	12.4	3.20	mod.	71	78.6	faint	49.2	„ „ before operation.
„ 26	840	neg.	2.88	faint	—	—	—	—	„ „ after „
„ 27	1340	15.8	8.40	neg.	114	71.9	faint	55.6	„ „ 1 gm. phlorizin subcut.
„ 28	1660	11.8	8.16	faint	—	—	—	—	„ „
„ 28	1190	4.0	2.04	„	—	—	—	—	„ „

On July 25 the ketosis seemed to be increasing, as should be anticipated. Under ether, silk ligatures were placed about both renal arteries, so as to occlude them as far as possible without total stoppage of the blood flow. Glycosuria ceased, and the nitroprusside reaction became almost imperceptible.

July 26, a third dose of phlorizin restored heavy glycosuria, but ketonuria remained absent or insignificant. There were no clinical symptoms of acidosis. The dog appeared well shortly after the operation, but developed gradually increasing malaise and weakness. Feeding was begun on July 29, but the appetite was poor. The cachexia led to death on Aug. 10.

Autopsy showed collapse and atrophy of extensive areas of the renal parenchyma, but no necrosis. The other viscera were negative grossly and microscopically.

## REMARKS.

Partial occlusion of the renal arteries seemed to act like partial occlusion of the veins in suppressing glycosuria and ketonuria. The return of glycosuria with continued absence of ketosis seems

to speak for some degree of independence between the two. The plasma sugar rose slightly; it is uncertain whether there is any difference between the artery and vein operations in this respect. The cause of death presumably comes under the heading of "uremia", as the symptoms resembled those following the removal of too much kidney tissue.

#### 4. PARTIAL LIGATION OF AORTA.

Dog G7 - 68 was a yellow and white male mongrel in good nutrition at a weight of 15 kg. Fasting and phlorizin were begun on July 8, as shown in table 7.

July 13, two heavy silk ligatures were placed on the aorta just distal to the renal arteries. The first ligature reduced the lumen of the aorta by about half, in order that the narrowing should not be so sudden as to give rise to erosion and hemorrhage. The second ligature, slightly below the first, was tied so tightly that only a small stream came through it,

TABLE 7.

*Dog G7 - 68.*

Date 1918	URINE					BLOOD PLASMA			REMARKS
	Vol. cc.	Sugar gm.	Total N gm.	D/N ratio	Nitro- prusside	Sugar mg. %	CO <sub>2</sub> Cap. Vol. %	Nitro- prusside	
July 8	400	10.56	3.80	2.77	neg.	—	—	—	Not fed. 1 gm. phlorizin subcut.
" 9	1100	22.00	10.23	2.15	faint	—	—	—	" " 1 gm. phlorizin subcut.
" 10	1320	40.60	—	—	mod.	—	—	—	" " 1 gm. phlorizin subcut.
" 11	700	22.47	—	—	heavy	—	—	—	" " 1 gm. phlorizin subcut.
" 12	950	33.70	10.20	3.30	mod.	—	—	—	" " 1 gm. phlorizin subcut.
" 13	200	11.40	2.30	4.95	heavy	83	44.3	neg.	" " Urine up to 10:00. p m.
	310	14.80	5.68	2.60	"	—	—	—	" " „ 10:00 p.m. to 12:00 m. July 14
" 14	775	31.12	11.36	2.73	mod.	109	32.8	mod.	" " 1 gm. phlorizin subcut.
" 15	525	26.10	18.60	1.41	"	—	—	—	" " Blood urea 35.7 mg. %
" 16	900	73.98	14.76	5.00	faint	66	43.3	—	Fed 300 gm. lung.
" 17	790	34.80	12.56	2.77	neg.	69	48.1	slight	

while the renal arteries were plainly distended with an extra volume of blood.

The result was a fall in the D :N ratios, until July 16, when the retained sugar seemed to be swept out. At the same time the nitroprusside reactions of the urine were not particularly diminished, and they became positive in the plasma where they had previously been negative.

## REMARKS.

The experiment was devised to try the effect of active or arterial hyperemia of the kidneys, in comparison with the passive or venous hyperemia produced by the partial ligature of the renal veins. In one way this experiment seemed to furnish a useful control, for although the D:N ratios fell as much as after venous ligation, there was no apparent clearing of the ketosis. Mainly, however, it was of interest that the additional blood flow through the kidneys did not cause any striking increase of either glycosuria or ketosis.

## 5. LIGATION OF URETERS.

*Dog G7-76*, a female mongrel weighing 11.2 kg. in good condition, was started fasting on July 16, 1918, and was given subcutaneous injections of phlorizin as shown in table 8. On July 20, the abdomen was opened under ether anesthesia, and both ureters were tightly ligated a little above their middle.

TABLE 8.

*Dog G7-76.*

Date 1918	URINE				BLOOD PLASMA			REMARKS
	Vol. cc.	Sugar gm.	Total N gm.	Nitro- prusside	Sugar mg. %	CO <sub>2</sub> Cap. Vol. %	Nitro- prusside	
July 16	585	41.4	—	neg.	—	—	—	Not fed. 1 gm. phlorizin subcut.
" 17	1330	47.0	—	mod.	—	—	—	" " 1 gm. phlorizin subcut.
" 18	1240	76.8	9.32	"	—	—	—	" " 1 gm. phlorizin subcut.
" 19	280	62.0	—	heavy	—	—	—	" " Urine up to 10:00 p. m.
" 20	850	21.4	10.08	"	85	—	faint	" " " " " 10:00 a. m.
" 20	60	0.6	—	faint	—	—	—	July 21
" 21	No urine	—	—	—	164	27.1	neg.	" " 1 gm. phlorizin subcut.
" 22	1550	neg.	1.35	neg.	185	55.7	"	" " Blood before removing ligatures.
					185	39.5	"	" " after removing ligatures.
								1 gm. phlorizin subcut.

By July 22, the dog was vomiting but not apparently near death. On that morning the abdomen was again opened under anesthesia and the ligatures removed from the ureters. The dog still appeared well except for moderate weakness on the morning of July 23, but death occurred at



about 4 P. M. In the interval 1500 cc. of urine was passed, containing only 1.35 gm. of nitrogen and no sugar or acetone.

REMARKS.

This form of kidney damage also abolished glycosuria and ketonuria. The hyperglycemia following the operation is noticeable, but there was no accumulation of acetone bodies in the blood. The plasma bicarbonate rose, perhaps because of combustion of acid compounds associated with the previous ketosis, and then fell at the end, probably because of retention of phosphoric acid. The experiment conforms with previous evidence that phlorizinized dogs can metabolize sugar when its excretion is prevented.

*Dog G7 - 82*, a female bull terrier in good condition weighing 15 kg., was subjected to fasting and phlorizin as shown in table 9.

On July 26, after ketonuria was well established, the abdomen was opened

TABLE 9  
Dog G7-82

Date 1918	URINE					BLOOD PLASMA			Remarks
	Vol. cc.	Sugar gm.	Total N gm.	D/N ratio	Nitro- prus- side	Sugar mg. %	CO <sub>2</sub> Cap. Vol. %	Nitro- prus- side	
July 24	1060	24.7	10.34	2.38	faint	—	—	—	Not fed. 1 gm. phlorizin
" 25	390	heavy	4.32	—	mod.	—	—	—	" " subcut.
" 26	190	6.7	3.40	1.97	"	127	—	slight	" " Blood 12:30 p.m.
	—	—	—	—	—	204	32.8	neg.	" " 11:00 "
" 27	No urine	—	—	—	—	213	48.1	"	1 gm. phlorizin subcut.
" 28	290	neg.	1.86	—	neg.	200	34.7	"	Not fed. " "

under the usual ether anesthesia and both ureters ligated near the bladder. The plasma nitroprusside reaction, which had been positive, became negative. The blood at 11 P.M. also showed a low plasma bicarbonate of 32.8 vol. %, possibly as a result of the preceding ketosis or the recent operation. Hyperglycemia was already present, and continued to the end. The plasma bicarbonate rose on July 27, but fell on July 28, presumably from accumulation of phosphoric acid.

The dog was depressed and slightly weak, but retained water. After the taking of the blood sample on July 28, the abdomen was again opened and the ligatures removed from the ureters. The dog appeared to recover

fairly well, but was found dead on the morning of July 29. The urine between operation and death was 290 cc., containing 1.86 gm. N but no sugar or acetone. Autopsy was negative except for the usual results of ureteral ligation.

REMARKS.

The renal injury resulting from ureteral ligation suppressed both glycosuria and ketonuria. The ensuing hyperglycemia might be interpreted as a retention of sugar, but acetone on the contrary disappeared from both blood and urine.

Cat B2-84, an adult female weighing 3 kg., was subjected to fasting and phlorizin injections as shown in table 10.

On the morning of July 22, after taking a blood sample, both ureters were doubly ligated. The cat at first appeared unchanged, but weakened

TABLE 10  
Cat B2-84

Date 1918	URINE				BLOOD PLASMA			Corp. Vol. %	Remarks
	Vol. cc.	Sugar gm.	Total N gm.	Nitro- prus- side	Sugar mg. %	CO <sub>2</sub> Cap. Vol. %	Nitro- prus- side		
July 16	63	7.4	—	neg.	—	—	—	—	Not fed. ½ gm. phlorizin subcut.
" 17	32	2.1	—	"	—	—	—	—	" "
" 18	30	2.7	1.23	"	—	—	—	—	" " ½ gm. phlorizin subcut.
" 19	59	3.0	—	slight	—	—	—	—	" "
" 20	52	2.3	—	mod.	—	—	—	—	" " ½ gm. phlorizin subcut.
" 21	150	3.6	2.90	"	—	—	—	—	" "
" 22	—	—	—	—	85	30.9	slight	32.1	" " Ureters ligated.¶
" 23	—	—	—	—	111	7.7	faint	28.6	" " ½ gm. phlorizin subcut. fed.

rather rapidly and died in the early morning of July 24. Autopsy showed nothing but the usual consequences of complete ureteral ligation.

REMARKS.

Comparison of the blood samples, one taken before operation on July 22 and the other in the late evening of July 23, shows the following results of the ligation of ureters. The plasma

sugar rose slightly. The plasma bicarbonate, which was rather low on account of ketosis, fell to a very low premortal value, supposedly because of phosphoric acid retention. Acetone bodies in the blood, as judged qualitatively by the nitroprusside reaction, did not accumulate on the stoppage of urinary excretion, but apparently diminished. The corpuscle volume fell slightly, but did not give an explanation of any of the above phenomena on the basis of blood dilution.

## 6. TOTAL OR PARTIAL NEPHRECTOMY.

*Cat B2 - 85* was an adult male weighing 2.7 kg. The observations with fasting and phlorization are shown in table 11.

TABLE 11

Cat B2-85

Date 1918	URINE				BLOOD PLASMA			Corp. Vol. %	Remarks
	Vol. cc.	Sugar gm.	Total N gm.	Nitro- prus- side	Sugar mg. %	CO <sub>2</sub> Cap. Vol. %	Nitro- prus- side		
July 16	50	6.17	—	—	—	—	—	—	Not fed. ½ gm. phlorizin.
" 17	52	6.72	—	neg.	—	—	—	—	" " " ½ gm. phlorizin.
" 18	42	5.90	1.81	"	—	—	—	—	" " " ½ gm. phlorizin.
" 19	67	3.89	3.28	slight	—	—	—	—	" " " ½ gm. phlorizin.
" 20	23	1.09	0.82	heavy	119	29.0	mod.	36.4	" " " Urine up to 3:45 p.m.
" 21	—	—	—	—	239	—	neg.	34.3	" " " ½ gm. phlorizin.
" 22	—	—	—	—	187	18.5	"	29.6	" " " ½ gm. phlorizin.

By July 20, heavy ketosis was indicated by urine and blood tests. Therefore at 3:45 on that afternoon, both kidneys were removed. The clinical result was a gradually progressing weakness. The cat was found dead on the morning of July 23.

## REMARKS.

The fall of plasma bicarbonate and of corpuscle volume belong among the ordinary results of nephrectomy. The essential effects with reference to the present problem were the hyperglycemia and the disappearance of blood acetone.

*Dog F6 - 91*, a female bull terrier, weighed 11.4 kg. in a slightly thin condition. July 15, 1918, the upper half of the left kidney was removed. Recovery was prompt, and the next day fasting and phlorization were



TABLE 12

Dog F6-91

Date 1918	URINE					BLOOD PLASMA			Remarks
	Vol. cc.	Sugar gm.	Total N gm.	D/N ratio	Nitro- prus- side	Sugar mg. %	CO <sub>2</sub> Cap. Vol. %	Nitro- prus- side	
July 16	585	13.1	—	—	—	—	—	—	Not fed. 1 gm. phlorizin.
" 17	480	16.5	—	—	mod.	—	—	—	" " " "
" 18	300	17.0	4.80	3.54	heavy	—	—	—	1 gm. phlorizin.
" 19	170	5.7	2.92	1.95	mod.	—	—	—	" " " "
" 20	53	1.6	0.84	1.90	heavy	77	43.3	slight	" " " "
" 21	470	12.2	4.05	3.01	slight	—	—	—	" " " "
" 22	740	8.7	6.08	1.43	neg.	80	42.4	faint	1 gm. phlorizin.
" 23	740	5.2	—	—	"	—	—	—	" " " "
" 24	380	3.8	3.00	1.27	"	—	—	—	1 gm. phlorizin.
" 25	650	heavy	6.30	—	"	91	31.9	neg.	Refuses food.
" 26	740	12.3	7.44	1.65	"	—	—	—	" " " "
" 27	945	slight	—	—	"	—	—	—	" " " "
" 28	—	neg.	—	—	—	135	46.2	neg.	Blood urea 100 mg. %.

begun, as shown in table 12. The dosage of phlorizin being small, the D:N ratio on July 19 and 20 was below 2; nevertheless, heavy ketonuria developed.

July 21, the entire right kidney was removed under the usual ether anesthesia. Presumably because of the anesthetic, the D:N ratio for this day was high, but the ketonuria fell abruptly. From July 22 onward, the urine still contained albumin, casts, blood and pus cells from the damaged left kidney, but no acetone. The D:N ratio was slightly lower than before the last operation. Hyperglycemia did not develop in the manner ordinarily found after partial ligation of the renal veins. The dog retained water but refused food and was unwell. The blood urea on July 28 was 100 mg. per 100 cc.

Death occurred on July 29, supposedly from uremia. Autopsy was negative except for the deficit in the left kidney.

#### REMARKS.

As animals ordinarily survive the removal of one and a half kidneys, it is possible that the renal injury from phlorizin was a factor in causing death in this case. The reduction of kidney tissue apparently lowered the D:N ratios and suppressed ketonuria.

*Dog G7-71* was a female puppy evidently between 2 and 3 months old, and weighing 1.75 kg. in a good nutritive state. July 13, 1918, a silk ligature was placed so as nearly to occlude the left renal vein, and the right kidney

was removed. The pup appeared lively after the operation, but refused food. Death occurred in the afternoon of July 17. Acetonuria, which seems to be an invariable occurrence in normal puppies with such fasting, remained completely absent in this animal. Urine was passed in average quantities and showed only slight albumin.

### 7. INJECTIONS OF ACETONE BODIES.

Dogs G7-70 and G7-73 were adult animals in comparable condition, except that the former weighed 4.2 kg. and the latter 5.2 kg. On July 13, 1918, both renal veins of G7-70 were almost occluded with ligatures in the usual manner. The urine contained albumin and red cells as usual, but the clinical recovery was satisfactory.

TABLE 13  
Dogs G7-70 and G7-73

Time  P.M.	BLOOD						Remarks
	Plasma Sugar mg. %		Corp. Vol. %		Plasma CO <sub>2</sub> Cap. Vol. %		
	G7-70	G7-73	G7-70	G7-73	G7-70	G7-73	
July 15, 1918							
12:10	98	84	46.5	50.0	74.8	57.6	12:11 two injections.
12:30	256	208	44.1	51.6	63.3	57.6	12:32 four injections.
1:10	303	313	33.6	48.3	87.1	63.3	1:12 one injection.
1:25	323	—	32.6	—	90.5	—	G7-70 autopsy blood.
1:30	—	286	—	43.8	—	65.3	1:35 G7-73 two injections.
3:45	—	156	—	54.3	—	52.8	
4:30	—	135	—	44.6	—	87.1	4:30 G7-73 autopsy.

July 15, the two dogs were given parallel injections of a solution of the sodium salt of acetoacetic acid of approximately N/7 strength. Each injection consisted of 20 cc. of the solution for dog G7-70 and 25 cc. for dog G7-73. The injections were given into the external jugular vein, and the number and time of them are shown in table 13.

Following each injection, the dogs showed dyspnea and drunkenness, the former being transitory while the latter became deeper. These symptoms were much more marked in dog G7-70 than in G7-73. After the injection at 1:10 P.M., the latter dog was conscious and only moderately intoxicated, while the former went into deep coma and died at 1:25 P.M. Two additional injections (of 25 cc. each) were then required to throw dog G7-73 into coma. There was then no tendency to recover; the animal remained unconscious, with increasing weakness and with occasional slight convulsions, until death at 4:30 P.M. The autopsies of both dogs were negative.

## REMARKS.

Analyses for acetone bodies in blood and urine would have been highly desirable, but were impossible for want of chemical help. The plasma bicarbonate in both animals rose, presumably because of combustion of most of the acetoacetic acid, leaving the sodium available. The corpuscle volume showed that hydremia was earlier and greater in dog G7 - 70, with renal stasis, than in the normal control. The main point of the experiment was to show the comparative toxicity of acetoacetic acid in such animals. As should be expected, the animal with renal impairment had no extra resistance, but on the contrary was more easily intoxicated, presumably because of the impaired power of excreting acetone bodies.

Dogs G7 - 86 and G7 - 88 were adult mongrels of comparable type, except that the former weighed 6.2 kg. and the latter only 5.3 kg. July 25, 1918, dog G7 - 86 was subjected to the usual operation of ligating both renal veins so as not quite to occlude them. Both dogs were started fasting on this day. (Table 14.)

TABLE 14  
Dogs G7-86 and G7-88

Time	BLOOD								Remarks
	Plasma Sugar mg. %		Corp. Vol. %		Plasma CO <sub>2</sub> Cap. Vol. %		Plasma Nitroprusside		
	G7-86	G7-88	G7-86	G7-88	G7-86	G7-88	G7-86	G7-88	
July 27, 1918 P.M.									
3.15	112	91	43.3	40.4	63.9	56.7	neg.	neg.	Inj. N/7 levo. <i>β</i> -oxybutyric acid.
3.20	—	—	—	—	—	—	—	—	
3.50									
4.00	116	105	46.2	35.8	45.3	48.4	neg.	neg.	Inj. N/7 inactive. <i>β</i> -oxybutyric acid.
4.30	—	—	—	—	—	—	—	—	
5.20	104	115	33.1	30.1	37.6	43.1	neg.	neg.	

July 27, the two were used for parallel injections of  $\beta$ -oxybutyric acid into the jugular veins. The first solution for the purpose was levorotatory  $\beta$ -oxybutyric acid, supplied through the kindness of Dr. I. Greenwald of the Montefiore Hospital, who had isolated it from diabetic urine. This was diluted to N/7 strength with physiological salt solution. Each injection in dog G7 - 86 was of 22 cc., while each in dog G7 - 88 was of 18 cc. Between



3:20 and 3:50 P.M. five such injections were given, making a total of 110 cc. in dog G7-86 and 90 cc. in G7-88. Dyspnea and depression in the latter animal were brief and slight, but in dog G7-86 they were more marked, and tenismus was present without diarrhea.

As the supply of the levorotatory acid was exhausted, a change was made to the commercial (inactive) variety. Between 4:30 and 5:15 P.M., eight injections were given to each dog, making a total of 176 cc. of N/7 inactive acid to dog G7-86, and 144 cc. to dog G7-88. The former dog developed very marked dyspnea and weakness, but never seemed to be in serious danger. The latter dog was much less affected. Dog G7-88 passed abundant urine during the experiment, but the loss of a considerable portion prevented measurement. Dog G7-86 passed no urine, and catheterization at 5:30 P.M. yielded only 18 cc. In both dogs, the nitroprusside and sugar tests of the urine remained negative. The plasma sugar likewise remained normal. The plasma bicarbonate fell more in dog G7-86 than in G7-88, presumably because of the poorer excretion of acid. Both dogs survived the experiment and were used subsequently for other purposes.

#### REMARKS.

The dog with partially ligated renal veins showed greater susceptibility to intoxication with  $\beta$ -oxybutyric acid than a normal dog. By comparison with the experiments in which acetoacetic acid was injected, the latter acid is seen to be much more highly toxic, as reported by previous authors.

*Dogs G7-60, G7-61 and G7-62* were adult animals of approximately 5 kg. weight and chosen for as close similarity as possible. All were kept in stock for a week on identical (bread and soup) diets before the experiments were begun. They were all subjected to partial ligation of both renal veins on July 4, 1918, and then were used for parallel butyric acid injections under different conditions on July 5. All recovered equally well from the operation and seemed about equally strong on the experimental day. The experiment is unfortunately deficient, as the analyses of both urine and blood have been lost.

Dog G7-60 was left normal except for the ligatures on the renal veins. Dog G7-61 received a subcutaneous injection of 0.5 gm. phlorizin after the operation and had heavy glycosuria on the experimental day (July 5). After the ligation of the veins in dog G7-62, the entire pancreas was removed before closing the abdomen, and this animal also had heavy glycosuria at the time of the experiments.

The purest commercial butyric acid was used, and diluted for the purpose of injection with 0.85 % NaCl solution. The injections were given into the exposed jugular veins, at a rather slow rate and uniformly in the three animals, so that each injection lasted about fifteen minutes. The effect of the injections was a transitory hyperpnea and more prolonged somno-

lence, differing in degree in the different animals. All three dogs excreted urine colored with blood.

Dog G7 - 60 showed the briefest dyspnea and the least somnolence. The nitroprusside tests of the urine remained negative.

Dog G7 - 61 exhibited moderate transitory dyspnea and the most pronounced somnolence of the series. Sleep was almost continuous, and at times amounted to unconsciousness so that the animal could not be roused. Nevertheless the picture was of sleep or anesthesia, not of diabetic coma. Nitroprusside reactions in the urine were negative before the injections, but quickly became positive and increased to heavy with the successive injections.

Dog G7 - 62 was intermediate in clinical symptoms between the other two. Dyspnea was brief, and there was continuous depression and sleepiness but never unconsciousness. Nitroprusside tests were negative at first and gradually became heavy after the injections.

July 6, all three dogs had recovered approximately their former condition, though the urine of all three was still blood-tinged. On this day they were used for parallel injection of N/7 levorotatory B-oxybutyric acid. The solution was prepared from the sodium salt of the acid which was kindly supplied by Dr. V. I. Issacson of the Montefiore Hospital, having been isolated by him from diabetic urine.

Dog G7 - 60 showed brief dyspnea and continued depression and somnolence after the first injection of 50 cc. Practically the same symptoms followed the second injection. The third injection was well borne till near the end, when there was acute respiratory distress. This subsided somewhat, but the dog remained dyspneic, collapsed, and dimly conscious. Instead of passing off as in normal dogs, the weakness increased and ended in death at 5:30 P. M., heart, respiration and consciousness apparently failing simultaneously. The great vessels, viscera, brain and meninges were deeply congested. The gross autopsy otherwise was negative. The kidneys were deeply congested but small, and microscopically showed only recent hemorrhages and congestion. The pancreas was normal.

Dog G7 - 61 showed approximately the same symptoms as G7 - 60, except the acute respiratory distress and collapse at the end. The injections were stopped merely because of the death of the other dog, when this dog was weak and drowsy but yet conscious and apparently able to stand more acid. The symptoms, however, became gradually worse instead of better, and the dog was found dead on the morning of July 7. No anatomical cause of death was found. The great veins and viscera were not particularly congested. Grossly the kidneys were normal in size and only moderately congested, and microscopically they showed somewhat less stasis and hemorrhage than in the preceding dog. The pancreas and its islands were normal.

Dog G7 - 62 showed similar symptoms but decidedly less marked than those of the other two dogs. The increasing weakness did not end fatally till 1 P.M. on July 7. The great veins, viscera, brain and meninges were moderately congested. The kidneys were not enlarged, but showed the usual signs of acute stasis grossly and microscopically.



## REMARKS.

The qualitative urine reactions indicated less susceptibility to ketonuria in dog G7 - 60, which was aglycosuric, than in the other two dogs, which were glycosuric. There was no uniform distinction demonstrable with reference to the lowering of plasma bicarbonate or toxic effects of the injection. The slightness of the intoxication in dog G7 - 60 with butyric acid, and the early fatality with  $\beta$ -oxybutyric, are both probably mere accidental differences as compared with the other two dogs. No satisfactory imitation of diabetic coma was obtained with either acid. A nephritic complication is believed to be particularly dangerous in human patients with ketosis, because it may prevent the usual large excretion of acetone bodies; but as dogs ordinarily excrete only small quantities of these acids after injections, it seems improbable that the renal ligations could have played any important part in increasing the intoxication from the injections. The chief points of interest in the experiment seem to be three: (1) The partial ligation of the renal veins does not oppose any absolute barrier to the excretion of acetone bodies; (2) The injection of butyric or  $\beta$ -oxybutyric acid can cause an excretion of acetoacetic acid (as judged by the Rothera reaction), and the tendency to such excretion is greater in animals whose carbohydrate reserves have been depleted by glycosuria, even though they were free from ketosis before the injection; (3) Poisoning with fatty acids may result fatally even after the acids themselves have been consumed, and some light may thus be thrown on deaths which sometimes occur from diabetic coma after the acetone bodies have nearly or completely disappeared.

## 8. PARTIAL AND TOTAL PANCREATECTOMY.

*Dog G7 - 33* was a male Newfoundland, aged about 8 years and moderately obese at a weight of 29.6 kg. June 20, 1918, silk ligatures were placed about both renal veins close to their entrance into the cava. Albuminuria, malaise and lack of appetite were present for the following few days, but by June 24 the dog was lively and hungry.

Three doses of phlorizin, as shown in table 15, resulted in fairly high glycosuria and D:N ratios, but no ketosis was in evidence qualitatively or quantitatively.

Beginning July 1 the dog was given a mixed diet and appeared entirely well, except for traces of albumin and a few red cells in the urine.

July 28, pancreatic tissue weighing 42.2 gm. was removed, leaving a



TABLE 15  
Dog G7-33

Date 1918	URINE				BLOOD PLASMA		Remarks
	Vol. cc.	Sugar gm.	Total N gm.	Nitro- prusside	Sugar mg. %	CO <sub>2</sub> Cap. Vol. %	
June 23	—	—	—	neg.	—	—	1 gm. phlorizin.
24	270	4.83	3.18	"	—	—	
25	895	23.76	12.5	"	—	—	1 " "
26	740	11.68	8.40	"	—	—	
27	1000	22.00	7.48	"	—	42.4	1 " "
28	no	urine		"			
29	825	19.62	11.16	"	—	—	
30	80	faint	—	"	80	48.1	

remnant estimated at 3.0 gm. about the main duct (1/15). Heavy glycosuria ensued, first on mixed diet and then on beef-lung. Beginning Aug. 8, 200 gm. suet was added to the diet daily. The urine continued to show heavy sugar and traces of albumin and red cells, but no acetone.

The experiment was interrupted by death on Aug. 14, which was shown by autopsy to be due to pneumonia. Moderate hydropic degeneration was present in the pancreatic islands. The kidneys showed chronic congestion with destruction of some glomeruli, atrophy of occasional tubules, and considerable cellular proliferation. Other viscera were normal.

#### REMARKS.

The fasting, the phlorizin dosage, and the height of the glycosuria and D:N ratios were sufficient for well marked ketosis in any dog with normal kidneys, as far as the writer has ever observed. The absence of ketosis therefore seemed to indicate a special "immunity" resulting from the ligation of the renal veins. The attempt to learn how such an animal would react with respect to diabetic acidosis and coma was frustrated by the accidental death.

*Dog G7-81*, a black and tan female weighing 7.25 kg. in good condition, was totally depancreatized on July 20, 1918. There was no infection and the dog retained fair liveliness and appetite. On July 24 and 25, olive oil and cream were taken willingly. The urinary acetone tests then became positive for the first time, though most or all of the fat seemed to be passed unchanged in the feces. (Table 16.)

July 26, the abdomen was opened by a new incision, and the peritoneum was found still free from infection. Both renal veins were ligated as tightly as possible without causing complete occlusion. The glycosuria seemed to be little if at all affected, but ketonuria ceased. The dog grew progressively weaker and died on July 29. Autopsy showed peritonitis.

TABLE 16

Dog G7-81

Date 1918	URINE					BLOOD PLASMA			Remarks
	Vol. cc.	Sugar gm.	Total N gm.	D/N ratio	Nitro- prus- side	Sugar mg. %	CO <sub>2</sub> Cap. Vol. %	Nitro- prus- side	
July 21	230	3.90	—	—	neg.	—	—	—	Not fed.
" 22	300	8.61	5.74	1.50	"	416	13.7	faint	" "
" 23	250	5.28	5.13	1.03	"	—	—	—	" "
" 24	100	heavy	2.41	—	"	—	—	—	Fed 100 cc. olive oil.
" 25	190	"	5.24	—	mod.	385	17.6	faint	Fed ½ pint cream and 50 cc. olive oil. Blood 12:45 p.m.
" 26	100	3.05	1.06	2.88	slight	—	—	—	
" 27	300	6.16	3.96	1.55	faint	475	74.8	"	Not fed.
" 28	270	3.06	1.06	2.89	neg.	1000	48.1	—	" "

## REMARKS.

The partial ligation of renal veins was followed by cessation of ketonuria as usual. It was of some interest that the results in phlorizinized dogs were thus duplicated in a depancreatized dog. Nevertheless, ketonuria in totally depancreatized dogs is such a slight and unreliable phenomenon that too much weight should not be laid on this occurrence.

TABLE 17

Dog G7-92

Date 1918	URINE					BLOOD PLASMA			Remarks
	Vol. cc.	Sugar gm.	Total N gm.	D/N ratio	Nitro- prus- side	Sugar mg. %	CO <sub>2</sub> Cap. Vol. %	Nitro- prus- side	
Aug. 4	440	14.0	9.05	1.54	heavy	92	51.8	faint	Not fed. 1 gm. phlorizin.
" 5	365	19.7	5.68	3.46	"	—	—	—	" "
" 6	560	18.7	6.78	2.76	faint	98	47.3	mod.	" " 0.5 gm. phlorizin.
" 7	540	16.4	11.52	1.42	mod.	—	—	—	" "
" 8	530	1.9	1.44	1.32	neg.	555	21.4	slight	" " Blood 8:30 a.m.
	—	—	—	—	—	910	29.0	neg.	Autopsy blood.

*Dog G7 - 92*, a French bulldog aged 7 years and weighing 15.5 kg. in excellent condition, on Aug. 2, 1918 was started fasting and was given 1 gm. of phlorizin subcutaneously. By Aug. 4 marked ketonuria was present, though the D:N ratios were not high. (Table 17).

Aug. 5, the entire pancreas was removed. Glycosuria and the D:N ratio increased greatly. Nevertheless the nitroprusside reaction of the urine became faint on Aug. 6, though it increased in the plasma. The blood sugar showed, as usual, that the hyperglycemia ordinarily to be expected with pancreatectomy was counteracted by phlorizin.

On the evening of Aug. 7, with ketonuria again increasing, the peritoneum was opened by a new incision under ether anesthesia, and was found clean and free from infection. Ligatures were placed on both renal veins, so as not quite to occlude them. The urine before and after operation of this day was mixed. By the morning of Aug. 8, acetone had disappeared from the urine, though a trace persisted in the plasma. By evening it had disappeared also from the plasma. The D:N ratio had fallen. The obstruction of the renal circulation ordinarily results in hyperglycemia in phlorizinized dogs, but here this influence was augmented by the pancreatectomy, so that an extremely high plasma sugar of 0.910 % was reached. By evening acetone had disappeared from the plasma.

By evening on July 8 the dog was very weak and dyspneic. The body was very hot, indicating fever, though no temperatures were taken. It is not certain that the low plasma bicarbonate was due to acidosis; it may possibly have been an accompaniment of the dyspnea from some other cause, especially in a moribund animal. Progressive weakness, without unconsciousness or coma, led to death at 11:30 P.M. In the autopsy, performed immediately, the viscera were found extremely hot. The liver was large and very fatty, as in typical depancreatized dogs. The kidneys were congested but nowhere necrotic, and their veins were still slightly patent. No peritonitis or other cause of death was found.

#### REMARKS.

Noteworthy incidental points are the suppression of diabetic hyperglycemia by phlorizin, and its restoration or augmentation by interference with the renal circulation. Here also, the D:N ratios fell; but, if it be granted that a totally depancreatized dog cannot utilize sugar, the main point of the experiment is that the disappearance of ketosis cannot be thus explained.

*Dog G7 - 96*, a brindle male mongrel aged 5 years and in good condition at a weight of 11 kg., began to fast on Aug. 5, 1918. Subcutaneous injections of 0.5 gm. phlorizin were given on Aug. 5 and 7. By Aug. 8 heavy ketonuria was present.

In a single operation under ether on July 9, the entire pancreas was removed and both renal veins were partially ligated. Hyperglycemia en-



TABLE 18  
Dog G7-96

Date 1918	URINE					BLOOD PLASMA			Remarks
	Vol. cc.	Sugar gm.	Total N gm.	D/N ratio	Nitro- prus- side	Sugar mg. %	CO <sub>2</sub> Cap. Vol. %	Nitro- prus- side	
Aug. 8	220	5.9	3.66	1.61	heavy	—	—	—	Not fed. 0.5 gm. phlorizin on Aug. 5 and 7.
" 9	1040	—	—	—	"	85	61.4	mod.	" " Operation.
" 10	940	24.6	9.56	2.57	"	250	—	"	" " "
" 11	260	3.75	1.80	2.08	slight	196	47.6	heavy	" " Blood 11:30 a.m.
	—	—	—	—	—	590	—	faint	Autopsy blood.

sued; the D:N ratios were slightly higher than before, and acetone reactions persisted in blood and urine. (Table 18.)

On Aug. 10 and 11, the dog grew weaker but had normal temperature. On Aug. 11, because of thirst and vomiting of all water taken, 400 cc. of 0.85 % NaCl solution was given subcutaneously. There was no dyspnea, and consciousness was retained up to death at 9 P.M.

At autopsy, the peritoneum was clean. The liver was only slightly fatty. The right kidney was congested as usual, but the left showed little congestion and was practically normal grossly and microscopically.

#### REMARKS.

This experiment opposes the preceding one, by showing that the ketosis of phlorization and pancreatectomy is not necessarily abolished by partial ligation of the renal veins. Probably, however, the reason is to be found in the looseness of the ligature of the left renal vein and the practical absence of stasis in this kidney.

#### DISCUSSION.

*Nature of phlorizin glycosuria.* — While the point of attack of phlorizin in the kidneys and the suppression of the glycosuria by renal injuries have long been familiar, there has been very little investigation of the sugar metabolism after such suppression. Apparently the organism becomes able to utilize the large quantities of glucose which were previously wasted in the urine, and presumably this fact holds good not only with the small doses used in the present experiment but also with the dosage required for "total" phlorization. Epstein and Baehr<sup>4</sup> concluded that

phlorizin stimulates glycogen formation after nephrectomy. A relation may exist between this fact and the hyperglycemia observed in the present experiments. Had there been facilities for proper study, it is not improbable that a rise of the respiratory quotient might also have been demonstrable. The carbohydrate metabolism seems still to be more or less abnormal, as indicated by the moderate hyperglycemia. But it is remarkable that the phlorizinized animal may show an apparent complete inability to burn sugar (not due to excessively low blood sugar, according to existing knowledge of the glycemia of such animals), and yet acquire the power to burn sugar or otherwise utilize it as soon as the kidneys are removed or damaged. Such observations confirm the intimate relation of phlorizin with the kidneys, but on the other hand oppose the interpretation of phlorizin poisoning as a purely renal phenomenon and demand an explanation adequate to cover the effects upon the entire organism.

*Relation to clinical conditions.* — The tendency to ketosis is known to differ in dog and man. One example is the relative immunity of the dog to fasting ketonuria; another is the fact that infection seems to augment acidosis in human diabetics, while infection in dogs, irrespective whether the glycosuria falls or not, commonly results in a reduction or disappearance of acetone bodies. In line with this difference is the suppression of ketosis (not only that accompanying glycosuria, but also the simple fasting ketosis of puppies) by renal injuries in the dog, while in man a nephritic complication has long been recognized as increasing the danger of diabetic coma. A number of tests in the Physiatrie Institute (some of them included in the recent paper by Modern<sup>5</sup>) have proved that patients with various renal and vascular diseases are fully subject to fasting ketosis and show none of the resistance created by renal injuries in dogs or puppies. The discrepancy, however, seems to be entirely explained by the above-mentioned difference between species. In two more fundamental respects the facts in man and dog can be harmonized. First, as shown by the injections of acetone bodies, the immunity of the dogs with renal injuries is not due to any increased power to destroy these substances, but on the contrary the impairment of excretion makes them more easily poisoned than normal dogs. Second, the widespread impression that coma is due merely to the accumulation of acetone bodies



in the blood and tissues has never been proved by analyses in diabetic patients or dogs. It seems to be untrue that the danger in complicated or uncomplicated cases can be estimated by the quantities of any or all of the acetone substances in urine or blood.

*Cause of disappearance of acetone.* — If it be granted that renal injuries cause in phlorizinized animals a fall of the D:N ratio, probably an increased storage of glycogen, and possibly a rise of the respiratory quotient, a complete explanation of the disappearance of ketosis will be furnished according to prevalent views on this subject. A few indications of the existence of an additional factor are worth noticing, as follows. First, high D:N ratios are not necessary for the production of ketosis. Investigators heretofore have obtained inconstant results in regard to ketosis, perhaps for the very reason that the "maximal" phlorization usually employed may either kill the animal in some other way or may injure the kidneys sufficiently to interfere with ketone production. Second, the D:N ratios after renal injuries sometimes fall only slightly, or at least remain sufficiently high that a continuance of ketosis should be expected. Third, renal injuries seem to suppress the ketosis of fasting puppies, even when there has been no glycosuria. Fourth, the effect seems also to be obtainable in depancreatized dogs, which can scarcely be expected to utilize sugar even after injury or removal of the kidneys (though this result is open to discussion, and when found may perhaps be explained by shock). Fifth, an increased power to destroy injected acetone bodies was not demonstrable in the animals with renal damage. Altogether, the possibility of a primary influence upon ketogenesis should be given experimental consideration before the phenomenon is accepted as purely secondary to a reduction of glycosuria.

#### CONCLUSIONS.

1. Various forms of renal impairment (partial ligations of the vena cava, renal veins or arteries, ligation of ureters, partial or total nephrectomy) abolish or greatly reduce ketosis in phlorizinized dogs and cats. Partial nephrectomy seems to be somewhat less effective than the other procedures which involve the entire kidneys.



2. Similar results are obtainable in the ketosis of fasting puppies and (less positively) in depancreatized dogs.

3. The phenomenon is not a mere blocking of acetone excretion, because ketonemia disappears simultaneously with ketonuria.

4. The animals with partially ligated renal veins seem to acquire no increased ketolytic power, as judged by intravenous injections of acetone bodies.

5. The changes in carbohydrate metabolism are among the most remarkable. The D:N ratios fall, hypoglycemia is replaced by hyperglycemia, and the animals are apparently able to utilize the sugar which otherwise would have been excreted. Nevertheless, there are some indications that the reduction of ketosis may not be solely due to reduction of glycosuria but that some primary influence on ketogenesis may also be involved.

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## EXPERIMENTAL STUDIES IN DIABETES.

## SERIES V.—ACIDOSIS.

## 8. THE INFLUENCE OF RENAL IMPAIRMENT IN PHLORIZINIZED DOGS.

BY FLORENCE B. SEIBERT, PH. D., AND FREDERIC T. JUNG, B. S.

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The following experiments were performed in order to repeat some of the work of the preceding paper under conditions permitting more accurate chemical control.

The dogs were kept in metabolism cages, without catheterization. All the analyses were performed by the usual standard methods. The phlorizin dosage was chosen so as to produce submaximal D:N ratiis, thus favoring longevity and the maintenance of a constant acidosis. The ligatures on the renal veins were placed so tightly that a considerable mortality resulted. A number of dogs which died within 24 hours showed complete clearing of acetone bodies in blood and urine, but only the experiments with longer survival have been selected for publication.

### TABLE 1

*Dog DD<sub>4</sub>.*

[illegible]



*Dog DD4*, a black and brown mongrel aged about 5 years and in good nutrition, was phlorizinized while fasting, with results shown in table 1.

After the taking of the blood sample on Aug. 11, both renal veins were ligated as tightly as seemed possible without stopping the blood flow completely. The kidneys immediately became swollen, tense and dark as usual. After operation the urine was bloody, the dog was weak, and death occurred at 7:40 P. M. on Aug. 13. Autopsy showed no sufficient anatomic cause of death, as the narrowed lumen of the renal veins was still patent, and the kidneys, though engorged, were not necrotic.

The table shows that the excretion of sugar and nitrogen was sharply reduced following the operation. This fall of the glycosuria and D:N ratios may perhaps account for the sudden fall of acetone bodies in urine and blood. Hypoglycemia was converted into marked hyperglycemia. The blood seemed to show a slight retention of urea and chloride. There was no retention of phosphoric acid to account for the fall of plasma bicarbonate. Therefore this fall, in view of the reduction of ketosis, is presumably to be attributed to shock or the moribund condition.

TABLE 2

*Dog DD5.*

Date 1922	Wgt. K.g.	URINE							BLOOD PLASMA (mg. %)							Phlorizin. gm.	
		Vol. cc.	Sugar gm.	N gm.	D/N	Acetone gm.	NH <sub>3</sub>	Alb.	Micros. exam.	Sugar	Urea	Chlorides	Acetone	Creatinine	CO <sub>2</sub> vol. %		PO <sub>4</sub>
July 30	18.8																2
31		320	—	—	—	neg.											2
Aug. 1		0	—	—	—	—											2
2		860	53.35	18.43	2.90	neg.											2
3		780	39.93	12.63	3.16	neg.											2
4		405	28.39	6.52	4.35	.66											2
5		345	18.59	6.69	2.77	faint											2
6		250	5.20	4.30	1.20	neg.											2
7		740	32.26	13.47	2.39	1.45											2
8	14.8	245	6.37	3.25	1.96	neg.											2
9		850	24.55	9.94	2.46	trace											2
10		680	24.00	3.04		.9112											2
11	13.9	480	14.44	6.7	2.15	.414			many cells, no casts	.057	32	597	85	.885			2
12		lost	—	—	—	—											—
13		835	36.57	15.85	2.30	.753	1.22	neg		.051	29	496	18	1.3	40	3.20	2
14		lost	—	—	—	—							45		24		—
Operation. Partial ligation of both renal veins with double linen ligature. Not much trauma.																	
15		1215	10.62	5.72	1.85	.186			Leucocytes and granular casts; pus and blood cells.	.168	32	498	16	1.2	34	5.37	2
16	15.0	345	neg.	0.33	0.00	.130				.103		589	neg	1.3		5.15	—



*Dog DD6*, a brown mongrel aged about 4 years and in medium nutrition, with phlorizin and fasting gave the results shown in table 3.

With the small dosage of phlorizin in proportion to the weight, both glycosuria and ketosis were unusually slight and sometimes absent. They were, however, continuous for some days preceding the partial ligation of the renal veins on Aug. 19. After the operation, the glycosuria was on the whole as heavy as before, but for some reason the nitrogen output was higher, so that the D:N ratios were reduced. The urine remained negative for acetone, whether because of the sugar available from the increased protein break-down or for some other reason is undetermined.

Other features are: (1) the gradual rise of plasma sugar after the operation, leading to a distinct hyperglycemia on Aug. 21, followed by a fall, so that by Aug. 24 the percentage was as low as before operation; (2) the absence of any important increase of chlorides, urea or creatinine; (3) a well marked transitory retention of phosphoric acid, as indicated by the percentage in the plasma; and (4) the absence of any reduction of plasma bicarbonate in connection with this retention.

The dog survived and was used for other experiments.

TABLE 4

*Dog DD7.*

Date 1922	Wgt. Kg.	Vol. cc.	URINE					BLOOD PLASMA (mg. %)							Phlorizin. gm.	REMARKS
			Sugar gm.	D/N	N gm.	NH <sub>3</sub> gm.	Acetone gm.	Sugar	Urea	Chlorides	Acetone	Creatinine	CO <sub>2</sub> vol. %	PO <sub>4</sub>		
Aug. 16	5.9														0.5	
17		185	2.15	2.06	2.50	neg.	.0115									
18		180	2.03	2.48	.818	trace	.194								0.5	
							.076									
19		355		8.84		3.327	.546	.086	23	517	47	1.26	27	416		
							.504									
20	4.8	310	4.76	.695	6.83	2.928	.543	.090	36	649	10	1.39	22	3.79		Partially ligated both renal veins. Also stripped fatty capsules. Quick operation. Not much trauma. Dog dead.
							.563									
21		240	2.54	1.22	2.08	.873	.213	.175	80	649	—	1.49	22	12.25		

*Dog DD7*, a black and white mongrel aged about 1 year and in good condition, was subjected to fasting and phlorizin so as to produce heavy



ketosis, as shown in table 4. On Aug. 20 the renal veins were ligated with silk as tightly as possible without stopping the circulation completely, and also the kidneys were stripped of their fatty capsules so as to exclude any possible collateral circulation.

The dog seemed in fair condition on Aug. 21, but died early in the morning of Aug. 22. Both kidneys were found congested as usual but not necrotic. Each weighed approximately 21 gm. (i. e., no extreme engorgement). Other findings were negative.

The chemical analyses of Aug. 21 show the usual sharp fall of ketosis and the usual rise of blood sugar. The plasma chloride did not exceed the pre-operative level, but there was a rapid retention of urea and phosphoric acid. The latter was accompanied by only a relatively slight fall of plasma bicarbonate, presumably because it was partly balanced by the clearing of the ketosis.

This brief experiment affords a good illustration that the fall of acetone excretion is not due to retention of acetone in the blood. On the contrary, there is a disappearance of acetone from both blood and urine. The question is still left open whether this result is purely secondary to the reduced glycosuria (or increased sugar utilization), or whether it is to any extent an independent effect of the renal ligation.

#### CONCLUSION

The reduction of ketosis after partial ligation of the renal veins is confirmed by the quantitative analyses of acetone bodies in blood and urine. The observations otherwise resemble those in the preceding paper.



## EXPERIMENTAL STUDIES IN DIABETES.

### SERIES V. — ACIDOSIS.

#### 9. — ADMINISTRATION OF ACETONE BODIES AND RELATED ACIDS.

By FREDERICK M. ALLEN AND MARY B. WISHART.

The attempt to throw light on the problem of diabetic coma by means of feeding or injections of the acetone bodies dates back to the earliest times of acidosis research, but nothing can be regarded as definitely established until new investigations are made with more modern and more complete methods.

#### ADMINISTRATION OF ACETONE.

The discovery of "acetonuria" naturally led to tests of the toxicity of acetone.

Frerichs<sup>1</sup> reported experiments by Salomon in which both healthy and diabetic persons received 20 or 25 gm. acetone daily for as long as 5 days, and dogs were given 25 gm. per day for longer periods, without clinical effects or demonstrable acetonuria. Though details are omitted, it must be supposed that these quantities of acetone were distributed in small divided doses through each day, in order to account for the negative effects.

Penzoldt<sup>2</sup> mentions that Kussmaul observed no symptoms from 6 gm. acetone per day in men. Penzoldt himself proved that intoxication, similar to that from alcohol, was produced in rabbits by subcutaneous injections of as little as 1 gm. of acetone, especially if an animal was placed under a bell-jar to prevent elimination through the breath. Inhalation of acetone likewise caused intoxication.

Tappeiner<sup>3</sup> administered acetone by inhalation to tracheotomized dogs, cats and rabbits, and gave a careful description of the resulting state of intoxication and anesthesia.

Albertoni<sup>4</sup> found that men taking 15 or 20 gm. acetone by mouth experienced either no symptoms or only a slight depression. In dogs, 1 gm. per kilo orally had no effect, but 4 gm. per kilo caused drunkenness like that from alcohol. The fatal dose was about 8 gm. per kilo, and one of



the dogs died only after 48 hours. A 5 kilo dog showed only transitory prostration from 6 cc. of acetone injected into the jugular vein; a 3.3 kg. dog showed more severe symptoms from the same dose but still recovered. Acetone was found to increase the saliva and gastric juice, to accelerate the pulse and lower the blood pressure, and to depress the nervous centers.

After an interval of 13 years, further experiments with acetone in dogs were performed by Schwarz,<sup>5</sup> who concluded that acetone is difficult to oxidize in the body. Fasting or carbohydrate feeding has no influence on the quantity metabolized, and the totally depancreatized dog disposes of acetone the same as the normal animal.

After another 13 years, Sollmann<sup>6</sup> employed acetone incidentally in a study of chronic intoxications of rats with a series of alcohols. He observed that "methyl and wood alcohol and acetone are markedly more toxic than ethyl alcohol"; also that "acetone, 1.8 cc. per kilogram per day as 2.5 per cent., is not fatal even after four months administration. Its effects on growth and food consumption are about the same as 2.5 per cent. methyl alcohol."

#### ADMINISTRATION OF ACETOACETIC ACID.

When it was early decided that acetone is not the cause of diabetic coma, and later discovered that only insignificant quantities of acetone are actually present in the body in acidosis, attention was turned to the other "acetone substances".

According to Frerichs,<sup>1</sup> Solomon gave dogs of 10 to 12 kg. as high as 20 gm. of acetoacetic ester daily for 8 to 10 days, with little or no symptoms and no demonstrable excretion in the urine. Healthy persons also ingested 20 gm. of the ester per day without results. Acetoacetic acid by mouth was similarly ineffective in men and dogs in doses of 10 gm. Quantities as high as 40 gm. per day caused "acetonuria" but no ferric chloride reactions. There was an aromatic odor of the breath, but no influence on the feelings or appetite. Dogs receiving 25 gm. of acetoacetic acid or its sodium salt subcutaneously were unaffected in behavior and showed no sleepiness or dyspnea. Acetonuria was present but the ferric chloride tests remained negative. The reported lack of symptoms with such doses subcutaneously seems to cast doubt upon this entire work, and render it doubtful whether the material administered actually contained any high proportion of acetoacetic acid.

Albertoni<sup>4</sup> performed experiments with acetoacetic ether and crotonaldehyde as well as acetone, but he arrived at no opinion concerning the cause of coma.

The main outcome of the experiments of Schwarz<sup>5</sup> was that the normal dog burns administered acetoacetic acid completely, but the depancreatized dog partly excretes it as acetone.

Passing reference may be made to the studies of Geelmuyden,<sup>7</sup> Maase,<sup>8</sup> Blum,<sup>9</sup> Dakin,<sup>10</sup> Neubauer,<sup>11</sup> and Marriott.<sup>12</sup> (Cf. also review by Porges<sup>13</sup>). These experiments involved the administration of acetoacetic,  $\beta$ -oxybutyric and other fatty acids to healthy and diabetic persons, and to normal and

phlorizinized dogs, but the purpose in view was merely the investigation of the course of fat metabolism, and particularly the demonstration that butyric acid gives rise first to acetoacetic and this secondarily to  $\beta$ -oxybutyric, and not *vice versa* as previously supposed. Dakin gave such doses as 12 gm. acetoacetic acid (as sodium salt) to a 4 kg. cat intravenously within 6 hours, 10 gm. to a 3 kg. puppy (death after 4 hours), 12 gm. to a 12 kg. dog in 6 hours, and the same to a larger dog in 4 hours. Marriott gave dogs of 5.4 to 7.9 kg. intravenous injections of acetoacetic acid equivalent to 6.17 gm. acetone, on the third day of fasting and phlorization. He also gave a 5 kg. sucking pig a subcutaneous acetoacetate injection equivalent to 4 gm. of acetone. Neubauer fed as high as 30 gm. of sodium acetoacetate to diabetics without injury.

Hurtley<sup>14</sup> published one of the best critical reviews of this question. He opposed the view, originated chiefly by Naunyn and Magnus-Levy, that  $\beta$ -oxybutyric acid, merely because it is quantitatively predominant, should be regarded as the essential cause of diabetic coma. He strongly defended the thesis that coma is a specific poisoning with acetoacetic acid. He pointed out that though there is only a difference of 2 between the molecular weights of acetoacetic and  $\beta$ -oxybutyric acid, the former is seven times as strong an acid as the former in base-combining power. He regarded the conversion of acetoacetic acid into  $\beta$ -oxybutyric acid as a protective mechanism, because the latter acid is weaker, less toxic, and it and its salts are very highly soluble. He assumed the failure of this mechanism, and the consequent accumulation of acetoacetic acid, to be one of the chief causes of coma. From the literature and from his own clinical studies he brought evidence to show that the relation between acetoacetic and  $\beta$ -oxybutyric acid (the so-called beta ratio) is variable, and that an absolute or relative rise of the acetoacetic factor is associated with coma. He expressed regret for the limited scope of his investigation, and to the lack of opportunity must doubtless be attributed the fact that he made no direct tests of the comparative toxicity of the two acids.

#### ADMINISTRATION OF $\beta$ -OXYBUTYRIC ACID.

The above mentioned authors<sup>8 to 12</sup> proved that administration of acetoacetic acid causes an excretion not only of the same acid but also of  $\beta$ -oxybutyric; while the latter acid, though with sufficiently large doses it may appear in the urine itself, does not give rise to any important acetoacetic excretion. Neubauer<sup>11</sup> thus gave a diabetic 30 gm. of l- $\beta$ -oxybutyric acid at one time without harm. Dakin<sup>10</sup> gave rabbits of about 1.7 kg. as much as 10 gm. of the inactive acid (neutralized with NaOH) by stomach or 3 gm. intravenously, likewise 8 gm. subcutaneously to a dog of 6 kg. Marriott<sup>12</sup> found that phlorizinized dogs metabolize the d-acid but only a part of the l-acid.

Sternberg<sup>15</sup> gave 5 gm. of  $\beta$ -oxybutyric acid per day to a neurasthenic woman orally for 5 days, without symptoms or ketonuria. He likewise fed a "severely" diabetic patient 5 gm. for 3 days and 10 gm. for 2 days, without clinical effects or urinary excretion. With the additional support



of animal experiments, he concluded that  $\beta$ -oxybutyric acid lacks the high toxicity of butyric.

Wilbur<sup>10</sup> injected rabbits intravenously with acids and their salts. He found that  $\beta$ -oxybutyric acid produced dyspnea, somnolence, convulsions and death in a manner not duplicated by equivalent doses of HCl. These symptoms were also produced by the sodium salt of  $\beta$ -oxybutyric acid. He regarded this as experimental evidence that the symptoms of diabetic acidosis are not due solely to lack of alkali.

The observations of Tschun-Nien<sup>11</sup> are mentioned below under butyric acid.

### ADMINISTRATION OF BUTYRIC AND OTHER ACIDS.

The earliest experiments with butyric acid, e. g. by Munk, Minkowski, and Araki, need not be reviewed in detail.

Mayer<sup>12</sup> found that subcutaneous injections of the sodium salts of formic, propionic, butyric and valerianic acids produce somnolence, sleep or coma, while no such symptoms result from equivalent doses of sodium chloride, acetate or lactate. Excluding formic acid because of its aldehyde character, he made the generalization that the narcotic action increases with the number of carbon atoms in the acids.

Binz<sup>13</sup> reported experiments of one of his students, showing that if normal dogs or cats receive 0.5 gm. per kg. of sodium butyrate subcutaneously or intravenously they fall asleep within 15 minutes, and with "sufficient dosage" this sleep passes into coma. Sodium chloride or acetate lacked this effect. He therefore favored the view that oxybutyric acid causes diabetic coma.

Sternberg<sup>14</sup> found butyric much more toxic than  $\beta$ -oxybutyric acid in experiments on cats and frogs.

Blum<sup>9</sup> observed that subcutaneous injections of butyric, caproic or iso-valerianic acid in dogs caused an excretion of acetone, acetoacetic and  $\beta$ -oxybutyric acid. In particular, this ketonuria resulted when the butyric acid was injected following a 40-minute chloroform narcosis, though the same animal previously had tolerated twice the dose without ketonuria. Etherization for the same period did not have the same effect as chloroform narcosis.

Ringer<sup>20</sup> gave subcutaneous injections of a series of acids in phlorizinized dogs, finding that those with uneven carbon atoms produce glucose while those with even carbons produce acetone bodies. Thus, valerianic and heptylic acids increased glycosuria, while butyric and caproic acids increased ketonuria. His doses of butyric acid were 10 gm. (as sodium salt) to a 13 kg. dog, and 20 gm. (in 2 doses) to an 11 kg. dog.

The experiments of Marx, Ehrmann, Esser, and Loewy have been the chief support of the doctrine that diabetic coma is a specific poisoning with acetone bodies. Marx<sup>21</sup> used puppies 6 to 12 weeks old, on the theoretical ground that young diabetics are most susceptible to coma. These puppies weighed a little above or below 5 kg., and received usually 6 gm. of sodium butyrate, at first by stomach, later and more effectively by intraperitoneal injection. The clinical results were an initial stage of excitement, followed



by various degrees of somnolence up to complete coma. Sugar feeding is said to have afforded some protection. There were no quantitative analyses, but acetone was demonstrated in the urine and blood by qualitative tests.

Ehrmann and Esser<sup>23</sup> studied the symptomatology more closely and made a detailed comparison with diabetic coma. The soft eyeball was thus found to be common to butyrate poisoning and diabetic coma. Oral and intravenous doses were used, and the latter sometimes caused convulsions. Slowing of the respiration (different from clinical coma) was avoided when the ethyl ester was used instead of the sodium salt of butyric acid. The dose necessary for coma in rabbits was 3.2 to 3.6 gm. per kg. intravenously. As emaciation did not alter the absolute dosage required, the acid was assumed to be a poison for the central nervous system, which does not change like the rest of the body with changes of nutrition. Quantitative analyses of the urine, blood and liver revealed only a trifle of acetone and acetoacetic acid and no  $\beta$ -oxybutyric. These were therefore excluded as the cause of the coma.

To show the specificity as opposed to a non-specific intoxication, Ehrmann<sup>23</sup> proved that though 3.6 or 3.7 gm. per kg. of sodium butyrate produces coma and death, doses of sodium isobutyrate up to 6.1 gm. per kg. cause no coma. An injection of 6.5 gm. of the isobutyrate caused coma, and one of 8 gm. caused death.

Loewy and Ehrmann<sup>24</sup> gave rabbits oral doses of sodium butyrate 6 times, of sodium valerianate once, and of sodium isobutyrate 3 times. They determined the  $\text{CO}_2$  content of the blood by Haldane's method, and the blood alkalinity by Loewy's titrimetric method. In the butyrate coma there was a marked lowering of the blood  $\text{CO}_2$ , but with doses insufficient for coma there was no lowering. The titratable alkali was moderately reduced in 2 cases, but was normal in 1 case even with coma present. Entirely similar results were obtained with isobutyrate, which did not cause coma. Therefore acidification of the blood was excluded as a cause of the coma. It was furthermore pointed out that animals poisoned with mineral acids show no typical coma, but only a terminal collapse occurring at a stage when the blood alkalinity and  $\text{CO}_2$  are much lower.

The work of Schun-Nien<sup>17</sup> is a recent continuation of the foregoing. He gave rabbits organic acids by stomach in dosage of 2.5 to 4.25 gm. per kg., and HCl similarly in dosage of 0.9 gm. per kg. Using a modified Barcroft method, he observed similar changes in the  $\text{CO}_2$  combining power, irrespective whether butyric,  $\beta$ -oxybutyric, isobutyric or hydrochloric acid was used. But coma was produced only by the butyric and oxybutyric acids, while the isobutyric and hydrochloric caused only depression and weakness, not unconsciousness. These facts, together with the failure of the clinical treatment of coma with alkali, are regarded as proving that acidosis, though present in coma, is not the essential cause, but the cause is rather a specific intoxication by acids related to butyric.

### ACETONE ANALYSES OF TISSUES.

The early estimations by Magnus-Levy, based only on levo-rotation, are now rejected as not sufficiently accurate.

Sassa<sup>25</sup> likewise determined the  $\beta$ -oxybutyric acid alone, but by a better method. The values in normal organs were extremely low, and in phlorizinized dogs and diabetic patients they were in general close to the figures for the blood. With the obvious exception of the kidneys, the highest analyses were found in the liver; for example, in one case 0.0595% in the kidneys, 0.0401% in the liver, 0.0359% in the lungs, 0.0336% in the blood, and 0.0314% in muscle.

Geelmuyden<sup>26</sup> performed complete analyses of acetone bodies in the organs of patients dead of diabetic coma. The findings were essentially similar to those of Sassa with  $\beta$ -oxybutyric acid. No striking differences in the ratio of acetoacetic to  $\beta$ -oxybutyric acid were established in the tissues as compared with the blood.

As the analyses of organs planned in connection with the present work could not be carried out, an attempt to search out isolated figures of this kind in the literature has been omitted. Such analyses, however, are lacking to a remarkable degree, and though the opportunity for them in human cases has become much restricted with the advent of insulin, there is a need of further work in this field.

#### PLAN OF INVESTIGATION.

To throw light on the question whether intoxication by the acetone bodies affords a single and sufficient explanation of diabetic coma, the most direct method seemed to consist in producing such intoxications in animals. The observations should include not only the symptomatic effects but also acetone analyses in urine and blood, to determine particularly whether the concentrations of either acetoacetic or  $\beta$ -oxybutyric acid in such experiments show any significant resemblance to those found in clinical coma cases or in the different forms of ketosis in animals investigated previously in this series. There is a possibility that normal animals, which can metabolize these acids actively, may not react to them like diabetics, whose power of disposal of them is evidently impaired; for example, the reduction of body alkali may not be identical, and the concentration of the acids in the tissues in proportion to the concentration in the blood may be different. The reduction of alkalinity can be partly imitated by giving the organic acids together with HCl. More important is the opportunity to administer the acids to animals with existing ketosis (diabetic dogs, phlorizinized dogs, fasting puppies), the material for this purpose being provided as shown in the preceding studies of this series. In addition to the injections of acetone bodies and the direct information on the coma problem, there is the opportunity to study fat meta-



bolism by injecting other low fatty acids which are the supposed precursors of the acetone bodies, and observing whether the metabolism in animals with existing ketosis shows impairment at all stages or only at the ketone stage. In all experiments analyses of the tissues were proposed in parallel with the blood, because of the importance for several problems.

The execution of this comprehensive program was so far prevented that the writer has doubted the desirability of publication. Even in its disappointing incomplete form, however, the investigation has developed a few new points and corroborated some previously known. Students of this subject will recognize that the work would have appeared especially important several years ago, prior to the recent extensive researches on ketosis; but this line of experimentation has still not been duplicated except in part by Schun-Nien,<sup>25</sup> and the present fragmentary publication therefore seems justified.

#### METHODS.

Only the fewest and simplest chemical tests could be performed. The plasma bicarbonate was determined by Van Slyke's method, and the plasma sugar by Benedict's method. Only qualitative tests were possible for the acetone bodies, except in a very few instances, when Van Slyke's quantitative method was used. The corpuscle volume was sometimes estimated, by standard centrifugalization in graduated tubes, as a crude index of dilution or concentration of the blood.

For intravenous injections, a small patch of each jugular vein was exposed in advance, and one vein was used for injections and the other for taking blood samples. The materials injected were all commercial articles of highest purity, except the acetoacetic acid, which was made from acetoacetic ether as directed by Beilstein, and the levorotatory  $\beta$ -oxybutyric acid, which had been prepared from diabetic urine by Dr. V. I. Isaacson of the Montefiore Hospital and was very kindly donated by him.

For purposes of comparison, experiments were performed on rabbits, puppies, and normal, phlorizinized and diabetic (depancreatized) dogs, but the series in each class is incomplete. The experiments are grouped according to the substances administered. Sometimes the acids were injected unchanged, but in the majority of instances they were first neutralized with



sodium hydroxide, and for convenience of distinction the name of the salt is here used; e. g., neutralized butyric acid is for brevity called sodium butyrate.

### EXPERIMENTS WITH ACETONE.

#### *Dog F6 - 74. Acetone by Stomach.*

*Dog F6 - 74*, a mongrel aged 1 year and with a normal weight of 5.2 kg., had been used for previous experiments and had a slight distemper. May 13, 1918, subcutaneous injections of pure acetone were given, with results shown in table 1.

TABLE 1  
Dog F6-74. May 17, 1918

Hour	BLOOD				Acetone injected subcut. cc.	Symptoms
	Corp. Vol. %	Plasma Sugar %	Plasma CO <sub>2</sub> Vol. %	Plasma Nitroprusside		
a.m.						
10.55	38.0	.101	53.8	neg.	—	
11.00	—	—	—	—	2	Slight excitement.
11.05	—	—	—	—	5	Increased excitement. Dyspnea.
11.08	—	—	—	—	5	Prostration; lying on side; slight dyspnea.
11.10	—	—	—	—	5	
11.12	—	—	—	—	5	Increased dyspnea.
11.15	34.8	.435	46.2	heavy	—	Semi-consciousness.
11.45	36.6	.525	44.3	"	—	
						Condition unchanged. Slow labored respiration.
p.m.						
12.05	—	—	—	—	5	Progressive weakness and unconsciousness.
1.00	43.4	.555	35.7	heavy	—	Death.

*Nitroprusside reactions* became immediately and continuously heavy in plasma and urine.

The *plasma bicarbonate* fell, and by the time of death was at the low level of 35.7 volumes per cent., corresponding to a severe stage of diabetic acidosis. Since acetone is not an acid, the only inferences open are that it gives rise to a production of acids (directly by transformation of the acetone, or indirectly by formation of lactic or other acids due to asphyxia), or else that the fall of blood alkali is compensatory for the pumping out of CO<sub>2</sub> by the hyperpnea.

The *clinical effects* consisted in dyspnea, excitement and drunkenness, passing gradually into semi-consciousness and finally unconsciousness. The picture, though somewhat suggestive of diabetic coma, more closely resembled alcoholic intoxication. In the autopsy, the venous engorgement found after injections of the lower fatty acids was lacking.

*Dog F6 - 70. Acetone Intravenously.*

*Dog F6 - 70*, a female mongrel weighing 10.5 kg., received intravenous injections of pure acetone as shown in table 2.

*Nitroprusside reactions* immediately became heavy in the plasma and urine, as should be anticipated. It was more surprising that these reactions were still positive on the following morning.

TABLE 2  
Dog F6-70. May 3, 1918

Time May 3 p.m.	BLOOD				Acetone injected cc.	Symptoms
	Corp. Vol. %	Plasma Sugar mg. %	Plasma CO Vol. %	Plasma Nitro- prusside		
4.20	37.7	84	72.9	neg.	—	Drunkenness, dyspnea.
4.25	—	—	—	—	15	
4.30	42.3	475	66.2	heavy	—	Increased dyspnea. Diarrhoea.
4.35	—	—	—	—	5	
4.50	40.0	—	55.7	heavy	—	Persistent dyspnea, depression, unsteadiness.
5.20	40.0	435	58.6	—	—	
6.30	52.0	—	62.4	"	—	Same condition. Respiration 60, very deep. Scanty bloody urine, giving heavy nitroprusside re- action. No thirst.
9.15	49.0	—	62.4	"	—	
May 4 a.m.	—	—	—	—	—	Slight dyspnea and depression. Urine still scanty and contains blood and acetone.
9.00	39.3	—	63.3	slight	—	

The *plasma bicarbonate* was temporarily lowered, supposedly as the result of pumping out of carbon dioxide by hyperpnea. The bicarbonate soon rose, however, to a high normal level, though the exaggerated breathing continued.

The *clinical effects* consisted essentially in drunken unsteadiness, drowsiness, and dyspnea. The dosage was not sufficient to produce unconsciousness. The symptoms were similar to those produced by the low fatty acids, but lasted longer. This fact, together with the persistence of nitroprusside reactions, seems to indicate that acetone is less easily disposed of by either combustion or excretion than the fatty acids.

*Dogs F6 - 76 and G7 - 30. Acetone Intravenously, Alone and with Sodium Bicarbonate.*

*Dog F6 - 76* was a female yellow mongrel which had recovered from some earlier experiments, and on June 18 weighed 7.7 kg. *Dog G7 - 30* was a similar mongrel weighing 6.3 kg. They were used on the same day for intravenous injections of acetone, the doses being proportioned to the body weight. In addition to the acetone, dog G7 - 30 received injections of 5 per cent. sodium bicarbonate solution, while dog F6 - 76 received similar quanti-

ties of 0.85 per cent. sodium chloride solution. The observations are shown in tables 3 and 4.

*Nitroprusside reactions* necessarily became heavy in the urine and plasma of both dogs. The long persistence of such reactions is noteworthy. Acetone is essentially a foreign substance in the body, and the fact that it

TABLE 3  
Dog G7-30. June 18-19, 1918

Hr.	BLOOD				URINE						Intravenous Injection	Symptoms
	Corp. Vol. %	Plasma Sugar %	Plasma CO <sub>2</sub> Vol. %	Plasma Nitro-Prusside	Vol. cc.	Sugar	Total N gm.	NH <sub>3</sub> -N, gm.	Alkalinity N/7 cc.	Nitro-Prusside		
p.m.												
5.10	46.5	.102	59.5	neg.	50	neg.	.10	.012	1.5	neg.		
5.15 to 5.30	—	—	—	—	—	—	—	—	—	—	100 cc. 20% acetone. 60cc. 5% NaHCO <sub>3</sub>	Drunkenness, stupor, salivation. No change.
5.45	50.5	—	36.6	heavy	—	—	—	—	—	—		
6.00	45.3	—	41.4	"	—	—	—	—	—	—		
6.05	—	—	—	—	—	—	—	—	—	—		
6.15	40.0	—	69.1	"	—	—	—	—	—	—		
6.35	35.8	.475	57.6	"	—	—	—	—	—	—		
6.40	—	—	—	—	—	—	—	—	—	—	20 cc. 20% acetone. 20cc. 5% NaHCO <sub>3</sub>	Moderate drunkenness and excitement, passing into stupor.
7.00	37.5	—	69.1	"	—	—	—	—	—	—		
7.30	—	—	—	—	—	—	—	—	—	—	20 cc. 20% acetone. 20cc. 5% NaHCO <sub>3</sub>	Deep stupor. Salivation.
8.00	48.2	—	74.8	"	—	—	—	—	—	—		
8.25	—	—	—	—	—	—	—	—	—	—	20 cc. 20% acetone.	Conjunctual reflex barely present. No dyspnea.
8.30	40.9	—	84.5	"	—	—	—	—	—	—		
10.00	41.5	.400	79.7	"	—	—	—	—	—	—		
10.05	—	—	—	—	—	—	—	—	—	—	20 cc. 20% acetone. 20cc. NaHCO <sub>3</sub> .	Conjunctual reflex lost.
10.20	—	—	—	—	—	—	—	—	—	—	20 cc. 20% acetone.	Complete unconsciousness, slight dyspnea.
10.50	45.2	.525	74.8	"	60	faint	.21	.006	2.6	heavy		
11.30	—	—	—	—	—	—	—	—	—	—		Dim consciousness.
a.m.												
1.50	46.9	.357	78.6	"	—	—	—	—	—	—		Slight recovery.
1.55	—	—	—	—	—	—	—	—	—	—	80 cc. 20% acetone.	Unconsciousness.
2.00	50.5	.715	76.7	"	18	faint	.078	.002	1.2	heavy		Death within 5 minutes.

seems to be metabolized slowly and with difficulty need not be surprising. It seems more remarkable that such a volatile substance should not be eliminated more rapidly, but in this respect it seems to resemble alcohol rather than ether.

The *plasma bicarbonate* was elevated as a result of the bicarbonate injections in dog G7-30, but there is no indication of death from alkalosis, unless the latter was masked by the acetone effect. The effect of the acetone itself was evidently a lowering of the blood alkali. There is no proof of acid formation on any such scale, and asphyxial hyperpnea seems to furnish a more probable cause for such a pseudo "acidosis". The fall of bicarbonate



was not sufficient to be regarded as a cause of death in dog F6 - 76.

The *clinical effects* were essentially drunkenness and stupor, out of proportion to the slight dyspnea. In their long duration these effects resembled alcohol intoxication rather than ether anesthesia. The most important point in the experiments is that the dog receiving sodium bicar-

TABLE 4  
Dog F6-76. June 18-19, 1918

Hour	BLOOD				URINE						Intravenous injections	Symptoms
	Corp. Vol. %	Plasma Sugar %	Plasma CO <sub>2</sub> Vol. %	Plasma Nitro-prusside	Vol. cc.	Sugar	Total N gm.	NH <sub>3</sub> -N gm.	Acidity N/7, cc.	Nitro-prusside		
p. m.												
5.30	40.7	.097	61.4	neg.	34	neg.	.19	.024	5.5	neg.		
5.35 to 5.50	—	—	—	—	—	—	—	—	—	—	120 cc. 20% acetone	Drunken excitement, salivation, stupor
6.15	40.0	—	44.3	heavy	—	—	—	—	—	—		
6.35	40.8	—	51.9	"	—	—	—	—	—	—	24 cc. 20% acetone. 24 cc. saline	Increased stupor
6.40	—	—	—	—	—	—	—	—	—	—		
7.05	42.8	—	69.1	"	—	—	—	—	—	—	24cc. 20% acetone. 24 cc. saline.	Increased stupor
7.35	—	—	—	—	—	—	—	—	—	—		
7.45	46.0	—	55.7	"	—	—	—	—	—	—	24cc. 20% acetone.	Conjunctival reflex barely present.
8.25	—	—	—	—	—	—	—	—	—	—		
8.30	37.0	—	50.0	"	—	—	—	—	—	—		
10.00	41.5	—	51.9	"	—	—	—	—	—	—	24 cc. 20% acetone. 24 cc. saline.	Crying and struggling as in recovery from ether. No such stupor as in dog G7-30.
10.05	—	—	—	—	—	—	—	—	—	—	24 cc. 20% acetone.	Unconsciousness. Recovers rather rapidly. Less stupor and more dyspnea than in dog G7-30.
10.20	—	—	—	—	—	—	—	—	—	—		
10.30	44.3	—	47.1	heavy	40	neg.	.16	.010	0	heavy		
a. m.												
12.40	44.2	.185	51.9	"	—	—	—	—	—	—		
12.45	—	—	—	—	—	—	—	—	—	—	120 cc. 20% acetone.	Dyspnea followed by unconsciousness and apnea. Recovery after artificial respiration.
1.00	46.8	—	47.1	"	—	—	—	—	—	—		
3.25	43.6	.526	38.5	"	—	—	—	—	—	—		
11.00	—	—	—	—	—	—	—	—	—	—	100cc. saline 60 cc. 5% glucose.	Semi-consciousness. Dyspnea and increasing weakness. Thirst manifest. Water vomited when given by tube.
11.15	39.0	.416	48.5	"	120	11.5 gm.	3.60	.026	9.0	heavy		Death.

bonate showed the more severe symptoms and the earlier death. It seemed evident that the alkali somehow increased the susceptibility to acetone intoxication.

The gross and microscopic autopsies were negative except for congestion in the great veins, viscera, brain and meninges. There was some "cloudy swelling" but no distinct fatty changes in the liver, kidneys and other organs.

## COMPARATIVE INJECTIONS OF DIFFERENT ACIDS.

*Rabbits A1 - 46, A1 - 48, A1 - 50 and A1 - 51. Butyric,  $\beta$ -oxybutyric, Acetoacetic and Hydrochloric Acids by Stomach.*

*Rabbits A1 - 46, A1 - 48, A1 - 50, and A1 - 51 were young gray adults of the same lot and the same weight. Fasting was begun on April 29, 1918, and on May 1 the weight of each rabbit was 1.3 kg. Solutions were prepared, each 75 cc. in volume, of the sodium salts of the following acids, calculated to represent N/7 strength of each acid: butyric, aceto-acetic, and  $\beta$ -oxybutyric (levo-rotatory, from diabetic urine). Also, 75 cc. of distilled water was taken, and to it was added the quantity of concentrated hydrochloric acid calculated as necessary to make N/7 strength. This same quantity of concentrated HCl was then added to each of the above solutions of organic acids. These acid solutions were then immediately given to the four rabbits by stomach tube, as follows: rabbit A1 - 46 received the  $\beta$ -oxybutyric, rabbit A1 - 48 received the plain hydrochloric, rabbit A1 - 50 received the aceto-acetic, and rabbit A1 - 51 received the butyric. The chemical observations are condensed in table 5.*

TABLE 5

Rabbits A1-46, A1-48, A1-50, A1-51

Rab- bit	Corpuscle Volume %				Plasma Bicarbonate Vol. %					Plas- ma Nitro- prus- side	URINE		Acid Administered
	May 1, 1918 Time P.M.			May 2  12.00 M.	May 1, 1918 Time P.M.				May 2  12.00 M.		Nitro- prus- side	Sugar	
	4.40	6.50	10.30		4.40	6.50	7.45	10.30					
A-46	32.6	—	—	—	60.5	—	—	—	—	—	+	—	$\beta$ -oxybutyric.
A1-48	30.2	32.6	26.1	—	53.8	38.5	—	30.7	—	—	—	—	Hydrochloric.
A1-50	30.0	28.6	26.1	19.8	61.4	40.4	—	38.6	70.0	++	+++	—	Acetoacetic.
A1-51	26.2	—	29.5	16.9	50.9	—	29.0	33.8	30.8	—	+	—	Butyric.

*Nitroprusside* reactions were naturally negative in rabbit A1 - 48, which received only hydrochloric acid. Rabbit A-51 seemed to produce a trifle of aceto-acetic acid or acetone from butyric acid, and rabbit A1 - 46 produced a greater quantity from  $\beta$ -oxybutyric. The heaviest reactions necessarily occurred in rabbit A1 - 50, which received aceto-acetic acid.

The *plasma bicarbonate* seemed to fall about equally in all the surviving animals, the differences being within accidental variations.

The *clinical effects* require separate mention for the different individuals.

Rabbit A1 - 46, receiving  $\beta$ -oxybutyric acid, exhibited immediate dyspnea and drunkenness, and died in 35 minutes. Large caseous glands in the thorax, probably tuberculous, seemed to be the explanation for the early death, rather than any disproportionate toxicity of the oxybutyric acid.

Rabbit A1 - 48, receiving the plain HCl, showed the greatest depression but the least dyspnea of all. All the doses were given about 5 P. M. The

respiration of this rabbit at 6:50 P. M. was 38, at 7:45 was 90, and at 10:30 was 40. It became increasingly deep and labored. Death occurred during the night.

Rabbit A1 - 50, receiving acetoacetic acid, responded with immediate drunkenness and dyspnea. The dyspnea gradually passed off. The animal lived with merely increasing weakness until 1 P. M. the next day, when death occurred with a slight convulsion. At this time the plasma bicarbonate was high and the nitroprusside reaction was negative in blood and urine.

Rabbit A1 - 51, receiving butyric acid, showed similar symptoms at first, but seemed strong and well when killed on the afternoon of May 2.

No animal exhibited unconsciousness or coma. Autopsy in all instances was negative. The odor of organic acid was strong in rabbit A1 - 46 but absent in A1 - 50 and A1 - 51.

BUTYRIC AND ACETOACETIC ACIDS INTRAVENOUSLY IN NORMAL, PHLORIZINIZED AND DIABETIC DOGS.

Dogs F6 - 95, G7 - 26, and G7 - 28 were fox terriers, having practically the identical weight of 4.3 kg. each. All three fasted, beginning June 18. Dog F6 - 95 served as the normal control. Dog G7 - 26 was totally depancreatized on the afternoon of June 17. Dog G7 - 28 received 0.5 gm. phlori-

TABLE 6  
Dogs F6-95, G7-26, and G7-28

Time June 20	URINE												BLOOD PLASMA						Intravenous injections.
	Volume cc.			Sugar gm.			Total N gm.			Nitro-prusside			Sugar,mg%			CO <sub>2</sub> Vol. %			
	F6-95	G7-26	G7-28	F6-95	G7-26	G7-28	F6-95	G7-26	G7-28	F6-95	G7-26	G7-28	F6-95	G7-26	G7-28	F6-95	G7-26	G7-28	
p. m.																			
2.30	—	—	—	neg.	h'vy	h'vy	—	—	—	neg.	neg.	neg.	143	322	90	53.8	48.1	50.9	20 cc. 2N/7 butyric acid.
3.15	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	20 cc. 2N/7 butyric acid.
3.25	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
3.45	—	—	—	—	—	—	—	—	—	—	—	—	137	384	89	37.6	40.4	34.7	
3.55 to 5.45	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	20 cc. 2N/7 butyric acid.
5.45	—	40	—	—	3.05	—	—	0.58	—	—	mod.	—	122	500	114	39.5	32.8	34.7	
10.00	43	61	375	neg.	2.18	10.96	0.51	0.55	4.68	neg.	faint	mod.	139	435	76	40.4	47.1	41.4	
June 21 12 noon	77	48	250	neg.	2.03	3.63	0.60	0.58	0.50	neg.	mod.	slight	—	—	—	—	—	—	
p. m.																			
4.00	24	45	78	"	1.29	2.96	0.48	0.92	1.14	slight	"	mod.	189	435	71	44.3	50.0	43.3	40 cc. N/7 aceto-acetic acid.
4.40	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
5.00	—	—	—	—	—	—	—	—	—	—	—	—	182	416	97	49.0	45.3	42.4	
5.20	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	40 cc. N/7 aceto-acetic acid.
5.35	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	40 cc. N/7 aceto-acetic acid.
5.55	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
11.00	114	77	132	beg.	1.03	3.20	1.17	0.94	1.43	mod.	heavy	heavy	111	455	128	66.2	58.6	50.9	
													92	370	115	65.3	56.7	—	



zin in oil suspension subcutaneously on June 17, 18 and 20. The three animals were then given identical intravenous injections of butyric acid on June 20 and of aceto-acetic acid on June 21. The chemical observations are shown in table 6.

*Nitroprusside reactions* were negative in all three animals prior to the injections. If the three had merely been kept on longer fasting without injections, it is presumable that the normal and the depancreatized dog would have developed no more than a trival ketosis, if any, while the phlorizinized dog would have developed a heavy or dangerous ketosis. Nevertheless the two dogs with glycosuria reacted practically the same to the injections, and differently from the normal dog. Both the glycosuric dogs excreted acetone bodies (as judged by the nitroprusside test) from the butyric acid, while this test in the normal dog was negative. With the aceto-acetic acid injections, the excretion as judged by this test was slight in the normal dog but considerable in the two glycosuric dogs.

The *plasma bicarbonate* followed no fixed rule, but it showed no more than a moderate or transitory fall, and there were no significant differences between the animals.

The *clinical effects* differed in the three dogs, but no specific differences between the butyric and diacetic acid were evident. The normal dog F6 - 95 exhibited only slight dyspnea and depression, and on the evening of June 21 appeared slightly weak but not seriously ill. Nevertheless this dog was found dead the next morning, with nothing in the autopsy to explain the result. The diabetic dog G7 - 26 was extremely prostrated and dyspneic from the butyric acid injections, but had recovered by the morning of June 21, and then stood the acetoacetic injections with somewhat less symptoms. The condition on the morning of June 22 was good, and death resulted only from a different experiment that afternoon. Dog G7 - 28 had polyuria from the butyric injections, in contrast to the other two dogs, and the symptoms were only slightly greater than those of dog F6 - 95. This dog was weaker than the others on the morning of June 21, and this weakness increased with the acetoacetic injections. Dyspnea was limited to a brief time following the injections; consciousness was not lost; but by the evening of June 21 the prostration was extreme. This dog also was found dead on the morning of June 22, and the autopsy was negative as usual.

#### *Dogs F6 - 64 and F6 - 65. $\beta$ -oxybutyric Acid and its Sodium Salt Intravenously.*

*Dogs F6 - 64 and F6 - 65* were male fox terriers weighing respectively 7.4 and 6.3 kg. They were used on April 22 for injections of commercial (inactive)  $\beta$ -oxybutyric acid into the jugular vein, the doses being 5 or 10 cc. per kilo of weight, and repeated in the usual manner. Dog F6 - 64 received the acid alone in N/7 solution. Dog F6 - 65 received the same solution, neutralized with NaOH so as to be faintly alkaline to litmus. The chemical observations are shown in tables 7 and 8.

*Nitroprusside reactions* remained negative in both urine and plasma of

TABLE 7  
Dog F6-64

Date	Time	Wgt. Kg.	URINE							BLOOD PLASMA					Intra- venous Injec- tions N/7 β-oxy- buty- ric acid cc.
			Vol. cc.	Sugar gm.	NH <sub>3</sub> - N gm.	Nitro- prus- side	Ace- tone & dia- cetic mg.	β-oxy, as ace- tone mg.	Total Ace- tone mg.	CO <sub>2</sub> Vol. %	Nitro- prus- side	Ace- tone & dia- cetic mg. %	β-oxy, as ace- tone mg. %	Total Ace- tone mg. %	
Apr. 22	p.m.														
	12.30	7.1	—	—	—	—	—	—	—	72	neg.	neg.	neg.	neg.	—
	12.35	—	—	—	—	—	—	—	—	—	—	—	—	—	37
	12.43	—	—	—	—	—	—	—	—	—	—	—	—	—	74
	1.00	—	—	—	—	—	—	—	—	—	—	—	—	—	74
	1.10	—	—	—	—	—	—	—	—	72	"	"	10.8	10.8	—
	1.18	—	—	—	—	—	—	—	—	—	—	—	—	—	74
	1.35	—	—	—	—	—	—	—	—	—	—	—	—	—	74
	1.45	—	—	—	—	—	—	—	—	—	—	—	—	—	74
	1.50	—	—	—	—	—	—	—	—	72	"	"	11.8	11.8	—
	12.30	—	220	neg.	0.04	neg.	neg.	252	252	—	—	—	—	—	—
	3.00	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	to	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	7.00	—	180	"	0.02	"	"	114	114	73.9	"	—	—	—	—
	a.m.	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	7.00	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	to	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	11.00	—	135	"	0.06	"	"	74	74	—	—	—	—	—	—

both animals. The quantitative analysis also revealed no formation of acetoacetic acid or acetone from the injected material, though the β-oxybutyric acid itself appeared in detectable quantities in the urine, and its

TABLE 8  
Dog F6-65

Date	Time	Wgt. Kg.	URINE							BLOOD PLASMA					Intra- venous Injec- tions N/14 β-oxy- buty- ric Acid, cc.
			Vol. cc.	Sugar gm.	NH <sub>3</sub> - N gm.	Nitro- Prus- side	Ace- tone and Diacetic mg.	β-oxy, as Ace- tone mg.	Total Ace- tone mg.	CO <sub>2</sub> Vol. %	Nitro- Prus- side	Ace- tone and Diacetic mg. %	β-oxy, as Ace- tone mg. %	Total Ace- tone mg. %	
Apr. 22	P. M.														
	12.35	6.3	—	—	—	—	—	—	—	80.5	neg.	—	—	—	—
	12.40	—	—	—	—	—	—	—	—	—	—	—	—	—	31.5
	12.55	—	—	—	—	—	—	—	—	—	—	—	—	—	63
	1.05	—	—	—	—	—	—	—	—	—	—	—	—	—	63
	1.10	—	—	—	—	—	—	—	—	73.9	neg.	neg.	20.2	20.2	—
	1.22	—	—	—	—	—	—	—	—	—	—	—	—	—	63
	1.39	—	—	—	—	—	—	—	—	—	—	—	—	—	63
	1.50	—	—	—	—	—	—	—	—	—	—	—	—	—	63
	2.00	—	—	—	—	—	—	—	—	74.8	neg.	neg.	20.9	20.9	—
	2.30	—	110	neg.	0.05	neg.	neg.	200	200	—	—	—	—	—	—
	3.00	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	to	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	7.30	—	120	"	0.03	"	"	100	100	83.4	neg.	—	—	—	—
	7.30	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	to	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	11.00	a.m.	100	"	0.04	"	"	41	41	—	—	—	—	—	—

concentration in the plasma was noticeably high. There seemed to be no significant difference between the two animals in these analyses.

The *plasma bicarbonate* was not lowered by the small doses of acid in dog F6 - 64, in blood samples taken ten minutes or longer after the injections. Also the alkali content of the injections in dog F6 - 65 had no distinct effect on the plasma alkali.

The *clinical effects* indicated no high degree of toxicity. There was no perceptible difference between the animals. Both were slightly depressed, but not dyspneic or in the least degree comatose, and both recovered easily. The trivial degree of the symptoms is of interest, in view of the fact that the  $\beta$ -oxybutyric acid in the plasma reached levels equal to those found in many comatose or pre-comatose diabetic cases.

#### ADMINISTRATION OF ACETOACETIC ACID.

##### *Rabbit A1 - 45. Acetoacetic Acid by Stomach.*

*Rabbit A1 - 45*, a young gray female, was started fasting on April 24; April 29, the weight was 1.1 kg., and the strength was still good. At 10:30 A. M. on this day, 100 cc. of N/7 aceto-acetic acid (in the form of the sodium salt) was given by stomach tube. The observations are shown in table 9.

*Nitroprusside reactions* were positive in both urine and plasma, as should be expected. The reaction in the plasma diminished before death.

TABLE 9  
Rabbit A1-45

April 29, 1918	BLOOD			URINE			Remarks
	Corp. Vol. %	CO <sub>2</sub> Vol. %	Nitro- prusside	Vol. cc.	Nitro- prusside	Dex- trose	
a.m.							Injection of aceto-acetate.
10.25	37.9	53.8	neg.	—	—	—	
10.30	—	—	—	—	—	—	
10.45	44.8	43.3	+ +	8	neg.	neg.	
11.25	53.4	39.5	+ +	—	—	—	
p.m.							
12.30	40.5	45.3	+	—	—	—	
3.00	30.0	51.9	+	—	—	—	
5.05	—	53.8	+	20	—	neg.	
10.15	26.2	60.5	+	—	—	—	

The *plasma bicarbonate* first fell and then rose. The rise can be explained by combustion of the organic acid, leaving the alkali free; but it is curious that the changes in bicarbonate were in inverse relation to the changes of corpuscle volume. Death from alkalosis seems to be excluded, as far as judgment is possible from the plasma bicarbonate.

*Clinical effects* appeared immediately in the form of weakness, drunkenness and dyspnea. The rabbit was quiet and depressed. It was easily pushed over on its side, and righted itself unsteadily and with difficulty. The respirations were 176 per minute, panting in character. These effects all increased, so that by 10:45 the rabbit was lying on its side, conscious but



weak, panting at the rate of 190 per minute. By 11:30 the rabbit insisted upon lying upright, though it could not stand. This condition remained practically unchanged up to 3 P. M., except that the respiration gradually diminished to 100 per minute. Throughout this time the odor of diacetic acid was strong on the breath. The rectal temperature was down to approximately 34° C. There seemed to be little further change up to 10:15 P. M., except a slowing of the breathing to 88 per minute. The rabbit seemed no weaker, though still unable to stand and noticeably cold to touch. The breath still had a diacetic odor, and salivation had appeared.

*Autopsy.* — The rabbit was found dead the next morning, and the autopsy was unsatisfactory because not fresh. It seemed negative as usual.

*Rabbits A1 - 42 and A1 - 47. Single Intravenous Injections of Acetoacetic Acid.*

*Rabbit A1 - 42*, a gray female adult weighing 1.4 kg., was given a single rapid intravenous injection of 56 cc. N/7 acetoacetic acid (slightly alkaline to litmus with NaOH), as shown in table 10.

TABLE 10  
Rabbit A1-42

April 27, 1918 P.M.	BLOOD				URINE		Remarks
	Corp. Vol. %	Sugar mg. %	Nitro- prusside	CO <sub>2</sub> Vol. %	Sugar	Nitro- prusside	
4.50	21.2	—	—	53.8	—	—	Injection
5.00	—	—	—	—	—	—	
5.10	23.6	—	++	63.3	—	+	
5.30	23.6	—	—	59.5	—	—	
6.00	27.6	—	—	56.7	—	—	
6.25	22.0	208	—	53.8	—	—	

Dyspnea and weakness became evident even during the injection. Following it, the rabbit lay semi-conscious on its side, with respirations 190 per minute. The breath had a sweetish odor. At 5:45 P. M. the respiration was still 150, the rectal temperature was 36.7° C., and the rabbit was able to sit up feebly. At 6:25 P. M. there was a single sudden convulsion ending in death.

Autopsy was negative except for the usual engorgement of the great veins. The blood and tissues had an indistinct aromatic odor, not indistinguishable with that of acetoacetic acid. Microscopic examination of the liver, kidneys and pancreas was negative.

From the single sugar analysis of the autopsy blood, it may be inferred that the hyperglycemia of 0.208% was caused by the acid injection. Urine was secreted in moderate quantity, and partly lost. Both it and the blood

plasma showed nitroprusside reactions following the injection, but these became negative before death.

TABLE 11  
Rabbit A1-47

May 1 1918	BLOOD PLASMA			URINE		Intravenous injections.
	CO <sub>2</sub> Vol. %	Nitro- prusside	Corp. Vol. %	Nitro- prusside	Sugar	
P.M.						
4.10	53.8	—	26.7	—	—	50 cc. N/7 Aceto-acetate.
4.23	53.8	+ + +	22.5	—	—	
5.30	59.5	+ + +	24.0	—	—	
6.15	63.3	+ + +	23.7	—	—	3 cc. N/7 HCl 3 cc. N/7 "
6.25	59.5	+ +	21.9	—	—	
6.40	55.7	+ +	19.6	—	—	
8.45	70.0	+	17.9	—	—	
10.35	84.4	+	16.7	+ + +	—	
May 2 1918						
11.00 a.m.	77.7	—	15.1	—	—	Autopsy.
1.00 p.m.	62.4	—	15.6	—	—	

*Rabbit A1-47*, a young gray female, was started fasting on April 29. On May 1 the weight was 1.2 kg. On that day 50 cc. of N/7 aceto-acetic acid, in the form of its sodium salt, was injected in a single dose into the jugular vein, as shown in table 11. The subsequent intravenous injections (each 3 cc.) of N/7 HCl are also shown in the table.

*Nitroprusside reactions* were naturally heavy for some hours in plasma and urine, but it is noticeable that they were entirely negative for half a day or more preceding death.

The *plasma bicarbonate* began to rise within less than an hour, evidently as a sign of combustion of the organic acid leaving the alkali free. To check the tendency to alkalosis, the two small injections of N/7 HCl were given at 6:15 and 6:25 P. M. The rise of the blood alkali nevertheless continued to the level of 84.4 volumes per cent at 10:35 P. M., but there was a fall to 62.4 volumes per cent at the time of death. Death from alkalosis seems to be excluded, as far as can be judged from the plasma bicarbonate.

The *clinical effects* of the sudden large intravenous dose were immediate prostration with only slight dyspnea. Within 15 minutes the respiration became deep and labored, 68 per minute. Within an hour the breathing was 90 per minute, of air-hunger type. The rabbit, which at first lay semi-conscious on its side, by this time was able to sit up feebly. The HCl injections produced very brief increase of respiration, but no perceptible lasting alteration. Respirations of the same type, 60 to 90 per minute, continued through the evening. Consciousness improved but not strength. By

the next morning the weakness was still greater, and death occurred quietly at 1 P. M. with stoppage of respiration, the heart continuing to beat while the thorax was opened. The autopsy was negative except for the usual venous engorgement.

*Rabbit A1 - 41. Successive Intravenous Injections  
of Acetoacetic Acid.*

*Rabbit A1 - 41*, a young adult albino female weighing 1 kg., on April 27, 1918, was given successive intravenous injections of N/7 acetoacetic acid in a solution faintly alkaline to litmus. Both jugulars were exposed easily at 10:25 A. M., and one of them was used for injections and the other for taking blood samples, as usual. As the analyses of urine and blood have been lost, only the clinical results can be described. A total of 55 cc. of the N/7 aceto-acetate was injected with fatal result within 2½ hours.

At 10:45 A. M., 10 cc. of the solution was injected into the jugular. Depression and dyspnea were immediate; the respirations were only 45 per minute, but deep and labored.

11 A. M., 5 cc. additional was injected. Hyperpnea, which had subsided, returned, and weakness was increased. The rabbit was unsteady and could be easily pushed over, but regained its feet actively.

11:30 A. M., 5 cc. additional was injected with similar results.

11:45 A. M., another 5 cc. was injected. The rabbit at first fell on its side, semi-conscious and breathing heavily, but after about a minute was able to sit up. Dyspnea gradually diminished but an appearance of weakness and illness continued.

12:05 P. M., 5 cc. more was injected. Condition seemingly unchanged.

12:20 P. M., 5 cc. more was injected. Dyspnea was still moderate, breathing 56 to 60 per minute, but weakness was marked and persistent, and resistance was practically absent.

12:45 P. M., another 5 cc. was injected. Symptoms slightly increased. The rabbit is still barely able to rise when placed on its side.

1:00 P. M., final injection of 5 cc. was given. The rabbit now lay on its side, practically in coma, the conjunctival reflex being practically the only sign of consciousness. The respiration was 62 per minute, but was diaphragmatic and feeble, bearing no resemblance to the breathing of diabetic acidosis.

1:10 P. M., death occurred by gradual failure of respiration and heart. Autopsy was negative except for the usual congestion of the right heart and great veins. No urine obtained. A *slight* nitroprusside reaction was given by the blood plasma in samples taken just *before* each injection. There is a note stating that the plasma bicarbonate in the autopsy blood was high.

ISOVALERIANIC ACID INTRAVENOUSLY IN A NORMAL  
AND A DIABETIC DOG.

*Dog G7 - 46*, a female mongrel weighing 6 kg., was totally depancreati-



zed on June 25, 1918. Two days later an experiment was performed with intravenous injections of 2N/7 isovalerianic acid, in parallel with the experiment on dog G7-52. The observations are shown in table 12.

TABLE 12

Dog G7-46. June 27, 1918

Hour P.M.	BLOOD				Injected 2N/7 iso- valerianic acid, cc.	Symptoms
	Corp. Vol. %	Plasma Sugar %	Plasma CO <sub>2</sub> Vol. %	Nitro- prus- side		
12.30	36.1	0.455	50.9	neg.	—	Dyspnea and depression.
12.40	—	—	—	—	50	
12.55	29.8	0.435	44.3	neg.	—	
1.00	—	—	—	—	25	Vomiting, diarrhea, dyspnea, semi-consciousness, limpness, salivation, scanty bloody urine.
1.15	—	—	—	—	25	
1.30	—	—	—	—	25	
1.45	—	—	—	—	25	
2.00	—	—	—	—	25	Continued dyspnea and stupor.
2.10	24.7	0.476	29.0	neg.	—	
5.20	28.4	1.050	40.4	"	—	
7.10	23.6	0.500	24.2	"	—	Death in coma.
8.00	25.7	0.358	16.6	"	—	

*Nitroprusside reactions* remained negative in blood and urine.

The *plasma bicarbonate* fell as the immediate result of the acid injections.

TABLE 13

Dog G7-52. June 27, 1918

Hour P.M.	BLOOD				Injected 2N/7 iso- valerianic acid, cc.	Symptoms
	Corp. Vol. %	Plasma Sugar %	Plasma CO <sub>2</sub> Vol. %	Nitro- prus- side		
12.35	37.1	0.097	57.6	neg.	—	Vomiting and diarrhea.
12.45	—	—	—	—	50	
12.58	33.9	0.170	32.8	neg.	—	
1.05	—	—	—	—	25	Dyspnea, depression, blood urine, salivation, moderate weakness.
1.20	—	—	—	—	25	
1.35	—	—	—	—	25	
1.50	—	—	—	—	25	
2.05	—	—	—	—	25	Slight depression, no dyspnea.
2.15	24.0	0.189	28.1	neg.	—	
5.25	26.7	0.106	52.8	"	—	
7.15	26.4	0.120	50.0	"	—	

In the long interval after termination of the injections, from 2:10 to 5:20 P. M., it rose somewhat, as is ordinarily the case when an injected organic acid is burned. But subsequently, without any further injections, it fell progressively, in a manner not explainable by dilution (as indicated by the corpuscle volume). This fall was perhaps a result of either the dyspnea or the moribund state, rather than a true expression of acidosis.

The *clinical effects* furnished an unusually close imitation of diabetic coma. The weakness and stupor were progressive, and the dyspnea was not transitory but continued to death. The usual venous engorgement was found at autopsy, but no close study of the organs was made.

*Dog G7 - 52* was a female mongrel weighing 4.8 kg. Fasting was begun on June 25, in preparation for use as a normal control for the totally depancreatized dog G7 - 46 on June 27. The results of the injections of isovalerianic acid into the jugular vein of the normal fasting animal are shown in table 13.

*Nitroprusside reactions* were negative in blood and urine.

The *plasma bicarbonate* was lowered immediately following the injections, but rose later, after the organic acid was presumably burned.

The *clinical effects* were similar to those in dog G7-46, but were much briefer and slighter. Though the normal dog was smaller, and thus received higher dosage per kilo, there was no unconsciousness and the dyspnea was neither great nor lasting. By evening this dog was nearly normal, and was subsequently used for hydrochloric acid injections, as described in the following paper (No. 10).

## EXPERIMENTS WITH BUTYRIC ACID.

### *Rabbits A1 - 37, A1 - 44 and A1 - 49. Butyric Acid by Stomach.*

*Rabbit A1 - 37*, a young adult gray female weighing 1.4 kg. received commercial butyric acid by stomach tube, as shown in table 14. The solutions were made alkaline to litmus with NaOH. The exposed jugular yielded blood very poorly in the period of greatest symptoms.

*Nitroprusside reactions* remained negative in the urine and no more than doubtful in the plasma. Butyrate poisoning therefore failed to give an imitation of clinical ketosis in respect to the formation of diacetic acid or acetone.

The *plasma bicarbonate* rose on April 24, as was to be anticipated from combustion of the butyric acid, leaving the alkali free in the body. For some reason, with the larger dose on April 25, it fell markedly, perhaps because of dyspnea, bleeding, or the moribund state. The urine was strongly alkaline on both days, showing alkalosis.

The *clinical effects* were great nervousness and restlessness, followed by depression with the smaller dose and semi-coma with the larger one. As the dyspnea was not due to acidosis, it may be attributed either to specific effects of the butyric acid or to the alkali (anoxemia?). The semi-consciousness and weakness were as marked as in dogs with diabetic coma, and the circulatory weakness also seemed similar. The jugular veins remained

TABLE 14  
Rabbit A1-37

Time Apr. 24, 1918	BLOOD PLASMA		URINE					Remarks
	CO <sub>2</sub> Vol. %	Nitro- prusside	Vol. cc.	NH <sub>3</sub> -N gm.	Alkalini- ty N/7 HCl. cc.	Nitro- prusside	Sugar %	
A.M. 11.20	76.7	—	—	—	—	—	—	11.30 A.M. given by stomach tube 50 cc. solution containing 4 gm. butyric acid neutralized with NaOH.
P.M. 12.40	65.3	neg.	—	—	—	—	—	Excitement followed by depression.
2.05	71.0	—	—	—	—	—	—	Moderate drunkenness and dysp- nea.
3.40	80.5	—	—	—	—	—	—	Respiration 128 per minute at rest.
6.10	84.4	—	—	—	—	—	—	Total urine since 10.00 A.M.
10.00	—	—	38	.006	4.0	neg.	.25	Symptoms diminished but present.
Apr. 25, 1918								Acts entirely well.
A.M. 10.00	90.3	neg.	—	—	—	—	—	10.05 A.M. given by stomach tube 50 cc. solution containing 7 gm. butyric acid neutralized with NaOH.
10.30	40.4	—	—	—	—	—	—	Excitement, restlessness, dyspnea began within 5 minutes, followed within another 5 minutes by paresis of hind legs, apathy, limpness.
10.45	31.9	—	—	—	—	—	—	Respiration 154 per minute, deep. Eye reflexes present.
11.05	30.9	—	—	—	—	—	—	Nearly unconscious. Deep pauseless respirations 124 per minute. Eye reflexes present. Fair imitation of diabetic coma.
11.15 Autopsy	29.0	—	16	.003	1.25	neg.	neg.	Death on withdrawal of 8 cc. of blood. Total urine since 10.00 A.M.

nearly empty, and blood samples were obtainable from them only by inclining the body with head downward. Death seemed to occur from the circulatory and general weakness.

*Autopsy* was negative for organic lesions. Notwithstanding the above-mentioned lack of flow in the jugular, there was engorgement of the caval

TABLE 15  
Rabbit A1-44

Time April 28, 1918	BLOOD			URINE			Remarks
	Corp. Vol. %	CO <sub>2</sub> Vol. %	Plasma Sugar	Vol. cc.	Dex- trose	Acet- tone	
5.20	35.6	50.0	—	24	neg.	neg.	6 gm. butyric acid (neutralized) by stomach tube.
5.55	34.5	44.3	—	5	"	sl. +	
6.20	40.0	44.3	192	—	—	—	
6.45	40.0	50.0	—	16	slight	mod.	20 cc. 20% glucose intravenously
7.00	—	—	—	12	heavy	sl. +	
7.17	—	—	—	—	—	—	20 cc. 20% " "
7.30	22.2	44.3	400	—	—	—	12 cc. 20% " "
7.55	—	—	—	18	heavy	faint	
8.00	18.3	33.8	475	8	"	neg.	
Autopsy							



veins, the viscera, and the right heart, while the left heart was empty. No odor of butyric acid was perceptible in the blood or tissues, and, in the unfortunate absence of analyses, this fact may have some importance.

*Rabbit A1-44*, a young gray male, was started fasting on April 24, and on April 28 weighed 1.3 kg. On this day 6 gm. of commercial butyric acid, neutralized with NaOH, was given by stomach tube. The solution and washings made a total of about 50 cc. The chemical results are shown in table 15.

*Ketosis* was indicated only by slight nitroprusside reactions in the urine, which were most marked about 1½ hours after the dose and became negative before death.

*Hyperglycemia* of 0.192%, together with slight glycosuria, was produced by the butyric dosage even in the fasting animal. The intravenous glucose injections greatly increased the sugar in both blood and urine, and may have facilitated the clearing up of the ketosis.

The *plasma bicarbonate* fell slightly at first, for unknown reasons, but at 6:45 P. M. had recovered its original level of 50.0 volumes per cent. The subsequent fall seems to be explained by dilution of the blood, caused by the glucose injections, as indicated by the corpuscle volume.

*Clinical effects* appeared in the form of slight depression, drunkenness and dyspnea (respirations 80 per minute) within 15 minutes after the dose. Within another 15 minutes the respiration had diminished to 60, and the rabbit lay on its side, only dimly conscious. There was slight diarrhea, and peristalsis was visible through the relaxed abdominal wall. At 6:10, the conjunctival reflex seemed to be the only evidence of consciousness, but when the blood sample was taken at 6:20 the rabbit woke up enough to resist slightly. The temperature was 36.8° C. There was no perceptible benefit from the glucose injections. The state of dim consciousness and advancing weakness continued to 8:00 P. M., when the animal stiffened out with a slight convulsion and died in apnea, the heart continuing to beat vigorously for a time even after the thorax was opened.

*Autopsy* was negative except for the usual venous engorgement. The brain and meninges were somewhat congested. There was no odor of

TABLE 16

Rabbit A1-49

Time May 1, 1918 P.M.	BLOOD			URINE		Remarks
	Corp. Vol. %	CO <sub>2</sub> Vol. %	Plasma Nitro- prusside	Sugar	Nitro- prusside	
4.25	—	38.5	—	—	—	4.0 gm. butyric acid (neutralized), in 50 cc. solution by stom- ach tube.
4.30	—	—	—	—	—	
7.25	29.1	57.6	++	—	—	
Autopsy	31.2	62.4	+	+	+++	

organic acids in the blood or tissues. The pancreas and kidneys were found microscopically normal.

*Rabbit A1 - 49*, a gray male, was started fasting on April 29, and on May 1 weighed 1.4 kg. After the taking of a blood sample at 4:25 P. M., the animal was given by stomach tube 50 cc. of water containing 4 gm. of commercial butyric acid neutralized to litmus with NaOH. The chemical observations are shown in table 16.

*Nitroprusside reactions* became strongly positive in both plasma and urine.

The *plasma bicarbonate*, which was rather low after the fast, rose markedly, but not to the point of a fatal alkalosis.

The *clinical effects* were prompt and increasing depression and weakness, with very slight dyspnea. The respiration did not exceed 61 per minute, but was slightly deeper than normal. There was no unconsciousness or coma at any stage. Death occurred with weakness and apnea at 7:45 P. M., the heart continuing for some minutes longer. The autopsy was negative as usual.

*Rabbit A1 - 43. Fasting and Phlorizin, and Butyric Acid Intravenously.*

*Rabbit A1 - 43*, a young gray female, weighed 1.2 kg. April 24, 1 gm. phlorizin in oil suspension was injected subcutaneously, and fasting begun.

April 28, injections of a normal solution of butyric acid, made neutral to litmus with NaOH, were given into the jugular vein, as shown in table 17. Three such injections were made, immediately following the taking of the blood samples at 11:10 and 11:20 A. M. and 12:20 P. M. These injections were individually 2, 6, and 18 cc. of the normal solution per kilogram, and in the aggregate 26 cc. per kilogram.

TABLE 17  
Rabbit A1-43

April 28, 1918 Time	BLOOD					URINE						Remarks
	Corp. Vol. %	CO <sub>2</sub> Vol. %	Acetone and Diacetic	$\beta$ -oxy- butyric mg. %	Total Acetone mg. %	Time	Vol. cc.	Acetone and Diacetic mg.	$\beta$ -oxy- butyric (as Ace- tone) mg.	Total Acetone mg.	Dex- trose	
a. m. 11.10	38.6	57.6	0	—	—	Before injec- tion.	—	trace	—	—	++	Injected 2.4 cc. N/1 butyrate.
11.20	33.4	53.8	trace	—	—	—	—	—	—	—	—	Injected 7.2 cc. N/1 butyrate.
11.30	33.4	69.1	"	—	—	—	—	—	—	—	—	
p. m. 12.20	35.0	72.9	0	24.1	24.1	11.35 to 12.40	12	neg.	—	—	++	Injected 22 cc. N/1 butyrate.
1.10	23.3	65.3	0	—	—	12.45	3	trace	—	—	+	
1.45	27.7	71.0	trace	—	—	12.55	5	"	—	—	+	
2.05	29.8	71.0	0	32.2	32.2	1.00 (12.40 to death, total)	11 19 6	" " 11.0	— — 47.8	— — 58.8	trace	Autopsy.

*Ketosis* was present at the beginning of the experiment (in consequence of the glycosuria and fasting) to the extent of a slight nitroprusside reaction in the urine, but not of sufficient degree for a positive nitroprusside



reaction or a lowering of the bicarbonate concentration in the plasma. The quantitative excretion of acetone bodies during the experiment remained trivial in comparison with the quantity of injected butyric acid. Both nitroprusside reactions and the quantitative figures for acetone in the plasma also remained slight.

The *plasma bicarbonate* rose during the experiment, evidently as a result of combustion of the organic acid leaving the alkali free, but this rise was not sufficient to indicate alkalosis as a cause of death.

*Clinical effects* were imperceptible after the first small injection of 2.4 cc. After the second dose (7.2 cc.) was injected, the rabbit appeared somewhat depressed, and the respiration seemed diminished rather than increased. The rate was 54 per minute, but the breathing was very shallow. The quantities of pale urine seemed to indicate a diuretic effect, especially after the last large injection. This injection (22 cc.) caused immediate prostration. The rabbit lay on its side, not unconscious but yet without any attention to surroundings or attempts to rise. Slow and shallow respiration was gradually replaced by dyspnea, so that by 12:45 P. M. the breathing was 186 per minute and panting in character. Weakness and drowsiness increased while dyspnea decreased. By 1:30 P. M. the respiration was 78 per minute, but deep and forcible, and the conjunctival reflex was barely obtainable. Death occurred quietly, from the progressive weakness, at 2:05 P. M.

*Autopsy* was negative except for the usual venous engorgement. The kidneys were wet. There was no recognizable odor of butyric acid in the blood or tissues.

### *Dog F6 - 63. Sodium Butyrate Intravenously in a Fasting Puppy.*

*Dog F6 - 63* was a male Newfoundland puppy about 3 or 4 months old, weighing 4.25 kg. Fasting was begun on April 20, in order to produce either acidosis or an increased tendency to acidosis. April 25, the weight was 3.6 kg., and ketonuria was still absent. On this day the animal was given intrajugular injections of butyric acid as shown in table 18. The solution was prepared by diluting commercial butyric acid to normal strength, and then neutralizing with NaOH to the point of faint alkalinity to litmus. The individual injections were either 5 cc. or 2.5 cc. per kilo.

*Nitroprusside reactions* quickly became heavy in the urine and moderate in the plasma. This ketosis was apparently heavier than occurs in adult dogs under these conditions. It may be mentioned incidentally that there was no butyric acid odor to the urine, the blood, or the tissues at autopsy.

The *plasma bicarbonate* rose as usual, presumably on account of alkali set free by the burning of the organic acid, but neither the analyses nor the clinical symptoms indicated death from alkalosis.

The *clinical effects* were persistent dyspnea, and drunkenness increasing to stupor and finally coma. The general picture seemed to be a fair imitation of diabetic coma.

The autopsy was negative grossly and microscopically, except for the



TABLE 18

Dog F6-63. April 25, 1918

Hour P.M.	URINE		BLOOD		Injection of N/1 butyric cc.	Symptoms
	Sugar	Nitro- prusside	CO <sub>2</sub> Vol. %	Nitro- prusside		
2.30	neg.	neg.	54.8	neg.	—	Slight depression and dyspnea.
2.35	—	—	—	—	18	
2.40	—	neg.	54.8	neg.	—	
2.45	—	—	—	—	18	Barking and struggling, followed by dyspnea and stupor. Respiration 22, temperature 38.6°, pulse 216.
2.55	—	—	59.8	slight	—	
3.00	—	—	—	—	9	
3.05	—	—	—	slight	—	Semi-consciousness.
3.10	—	—	—	—	9	
3.20	—	—	60.5	mod.	—	
3.22	—	—	—	—	18	Stupor, crying and dyspnea.
3.27	—	—	66.2	mod.	—	
3.28	—	—	—	—	9	
3.30	—	—	—	—	9	
3.35	—	—	64.3	mod.	—	
3.38	—	—	—	—	18	
3.50	neg.	heavy	68.1	mod.	—	
3.53	—	—	—	—	18	
4.00	—	—	70.0	mod.	—	Respiration 26, temperature 38.1°, pulse 180. Conjunctival reflex still present. Blood-tinged feces.
4.05	—	—	—	—	18	
4.10	—	—	72.9	mod.	—	
4.13	—	—	—	—	18	Pulse 165, respiration 32, full and deep. Abundant clear pale urine. Pulse 144, becoming weaker. Respiration 28, less deep.
4.20	neg.	heavy	72.9	mod.	—	
6.00	—	—	84.4	"	—	
7.20	neg.	heavy	61.4	"	—	Coma and dyspnea. Respiration 40, temperature 39.9°, pulse 220. Death.

usual congestion in the great veins and viscera, and noticeably in the brain and meninges.

*Dog F6 - 62. Sodium Butyrate and Butyric Acid Intravenously in a Fasting Phlorizinized Dog.*

*Dog F6 - 62*, a female mongrel weighing 17.6 kg., was started fasting on April 21, 1918. Subcutaneous injections of 1 gm. phlorizin were given on April 22, 23, 24 and 27. The record in table 19 is a good example of ketosis in a fasting phlorizinized dog, the excretion of approximately 7 gm. of total acetone on April 27 being exceptionally high and easily comparable with the ketonuria of human diabetics in proportion to body weight. On May 2, the dog was given intravenous injections of 2 gm. commercial butyric acid (neutralized with NaOH) and of 3 gm. butyric acid (not neutralized). On May 3 a single intravenous injectiton of 250 cc. N/7 acetoacetic acid in the form of the sodium salt was given.

*Ketosis.*—Unfortunately, part of the urine on both May 2 and 3 was lost, so that the quantitative changes in ketonuria could not be observed as planned. With the butyric acid, there was some increase of the nitroprusside color in the plasma but not in the urine. With the sodium acetoacetate the reactions became heavy in blood and urine.

TABLE 19

Dog F6-62

Date 1918	Time	Wgt. Kg.	URINE							BLOOD PLASMA			Remarks
			Vol. cc.	Sugar gm.	Total N gm.	Nitro- prus- side	Acetone and Diacetic acid, mg.	$\beta$ -oxy (as Ace- tone) mg.	Total Acetone mg.	Sugar mg. %	CO <sub>2</sub> Vol. %	Nitro- prus- side	
Apr. 21	—	17.6	175	neg.	11.00	neg.	trace	70	70	—	—	—	Not fed.
" 22	—	—	Urine	lost	—	—	—	—	—	—	—	—	" " 1 gm. phlorizin subcut.
" 23	—	—	360	5.74	7.36	"	12	20	32	—	—	—	" " "
" 24	—	—	875	40.00	12.33	heavy	600	130	730	—	52.8	—	" " "
" 25	—	—	700	25.50	8.12	"	310	650	960	—	—	—	" " "
" 26	—	—	1800	41.40	14.40	"	1060	1520	2580	—	51.9	heavy	" " "
" 27	—	—	3700	61.80	17.76	"	2555	4440	6990	—	—	—	" " "
" 28	—	—	2955	30.00	12.90	"	510	3300	3810	—	46.2	"	" " "
" 29	—	14.5	4000	35.60	10.80	mod.	520	200	720	96	62.4	—	" " "
" 30	—	—	3500	24.20	11.20	"	810	980	1790	—	51.9	—	" " "
May 1	—	—	3470	26.30	9.10	"	880	2170	3050	—	51.0	—	" " "
" 2	—	3.5	1565	15.02	—	"	—	—	—	85	50.0	—	" " Butyric acid injection.
" 3	—	—	3450	7.91	—	heavy	—	—	—	93	59.5	faint	" " N/7 aceto- acetate injection.
" 4	—	—	2800	neg.	4.20	slight	neg.	110	110	—	81.5	—	" " "
May 2	a. m.	—	—	—	—	—	—	—	—	85	50.0	slight	Injected 2 gm. bu- tyric acid in 8% so- lution, neutralized.  Injected 3 gm. bu- tyric acid in 8% so- lution, neutralized.
	11.00	—	—	—	—	—	—	—	—	—	—	—	
	p. m.	—	—	—	—	—	—	—	—	—	—	—	
	4.15	—	690	3.73	—	mod.	—	—	—	86	59.5	—	
	4.20	—	—	—	—	—	—	—	—	—	—	—	
	4.35	—	—	—	—	—	—	—	—	94	57.6	"	
	4.40	—	—	—	—	—	—	—	—	—	—	—	
	4.55	—	—	—	—	—	—	—	—	106	46.2	mod.	
	6.30	—	—	—	—	—	—	—	—	—	65.3	—	
May 3	7.00	—	300	2.07	—	mod.	—	—	—	—	—	—	Injected 250 cc. N/7 aceto-acetate.
	10.30	—	155	2.47	—	"	—	—	—	79	49.0	"	
	p. m.	—	—	—	—	—	—	—	—	—	—	—	
	2.07	—	260	1.59	—	faint	—	—	—	93	59.5	faint	
	2.40	—	—	—	—	—	—	—	—	—	—	—	
	2.55	—	—	—	—	—	—	—	—	—	65.3	neg.	
	5.07	—	—	—	—	—	—	—	—	111	76.7	"	
	6.15	—	990	2.00	—	heavy	—	—	—	—	—	—	
	9.15	—	270	0.72	—	"	—	—	—	—	78.6	heavy	

*Clinical effects.*—As a result of the fasting and phlorizin, the dog was showing weakness and apathy which are the early warnings of coma, though the plasma bicarbonate had not yet fallen. The chief purpose of the experiment was to learn whether an animal in such a state of glycosuria and ketosis becomes extremely sensitive to the lower fatty acids. Aside from the theoretical interest, there was a question whether this might afford an easy and sure means of producing an imitation of diabetic coma. The butyric injections on May 2 produced the usual depression and dyspnea, somewhat more marked than in a normal dog, but only transitory and not progressive. Consciousness was not lost. The acetoacetate injection on May 3 had very little perceptible effect, not supporting the idea of any extreme toxicity of acetoacetic acid in this condition. The dog subsequently regained complete health.

BUTYRIC ACID INTRAVENOUSLY IN NORMAL, PHLORIZINIZED  
AND DEPANCREATIZED DOGS.

*Dog G7-27* was a female toy bull terrier weighing 4.6 kg., and was totally depancreatized on June 17, 1918. *Dog G7-29* was a female yellow mongrel weighing 4.4 kg., and was given subcutaneous injections of 0.5 gm. phlorizin on June 17 and 18.

This dog was senile, obese and asthmatic, and such animals are known to react badly to pancreatectomy. Though there was no peritonitis, the result of the operation was prostration instead of glycosuria. The plasma sugar analyses show that hyperglycemia was also comparatively slight, i. e., the absence of sugar excretion was not due entirely to renal impermeability, though the threshold seemed to be high. This dog was intentionally used to test the susceptibility to ketosis from acid injections in a totally diabetic animal without glycosuria.

*Dog G7-31* was a black-and-tan female weighing 3.7 kg., and was used as a normal control. All three dogs fasted beginning June 17, and received injections of butyric acid into the jugular vein on June 19. The observations are shown in table 20.

TABLE 20  
Dogs G7-27, G7-29, and G7-31. June 19, 1918

Time P.M.	BLOOD PLASMA						URINE									Intravenous Injections.
	Sugar mg. %			CO <sub>2</sub> Vol. %			Vol. cc.			Sugar gm.			Nitro-prusside			
	G7- 27,	G7- 29,	G7- 31.	G7- 27,	G7- 29,	G7- 31.	G7- 27,	G7- 29,	G7- 31.	G7- 27,	G7- 29,	G7- 31.	G7- 27,	G7- 29,	G7- 31.	
5.00	172	99	112	67.2	59.5	64.3	—	—	—	—	—	—	—	—	—	40 cc. 2N/7 butyric acid.
5.10	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
5.25	192	98	106	48.1	60.5	32.8	—	—	—	—	—	—	—	—	—	40 cc. 2N/7 butyric acid. G7-31 died immediately fol- lowing injection. Autopsy blood.
5.40	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
5.45	—	—	125	—	—	24.2	—	—	4	—	—	0	—	—	hvy.	4 injections 20 cc. 2N/7 butyric acid.
6.00 to 6.15	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
6.20	222	111	—	40.4	37.6	—	—	—	—	—	—	—	—	—	—	
10.15	179	55	—	60.5	lost	—	88	122	—	0	14.4	—	neg.	mod	—	

*Nitroprusside reactions* were negative in all three animals before the injections, but became positive in the normal dog, and still heavier in the phlorizinized dog. They remained negative in the depancreatized dog, presumably on account of either renal impermeability or the general metabolic failure.

The *plasma bicarbonate* fell temporarily following the acid injections, and the disproportionate fall in dog G7-31 was presumably one factor in the early death of this animal.

The *clinical effects* took the usual form of transitory dyspnea and lasting depression. Dog G7-31 died in acute dyspnea immediately following the second injection, which probably was given too rapidly. But the smallest



dog of the three had intentionally been chosen for the normal control, with the idea that it might have a higher resistance than either the diabetic or the phlorizinized dog, and the higher dosage in proportion to weight may have been the chief cause for both the disproportionate fall of plasma bicarbonate and the early death. Dog G7 - 29 at the end of the experiment showed no dyspnea and only slight depression, and recovered completely. The depancreatized dog G7 - 27 at the end of the experiment had greater weakness but no dyspnea. By the morning of June 20 all effects of the injections seemed to have passed off, and death occurred only on June 21, evidently from the diabetic condition alone, still without any glycosuria.

*Dogs G7 - 63, G7 - 65 and G7 - 66. Comparative Injections of Butyric Acid and Sodium Butyrate Intravenously.*

Dogs G7 - 65 and 66 were obese female mongrels, weighing respectively 8.4 and 8.6 kg. and very similar in all respects. They had been in stock for one week on oatmeal diet, and were used on July 7 for comparative injections of butyric acid and sodium butyrate. Commercial butyric acid was diluted to N/7 strength, and the solution was divided into two parts. One part was used directly for the injections into dog G7 - 65. The other part was neutralized to faint alkalinity to litmus with NaOH and was then used for the injection into dog G7 - 66. (Tables 21 and 22).

TABLE 21  
Dog. G7-65

Time July 7, 1915	BLOOD		URINE					Injections of N/2 butyric acid, cc.	Remarks
	Corp. Vol. %	CO <sub>2</sub> Vol. %	Vol. cc.	Nitro- prus- side	Total N gm.	NH <sub>3</sub> -N gm.	Alka- linity cc. N/7)		
p. m.									
3.20	53.8	52.4	—	—	—	—	—	—	
3.25	—	—	—	—	—	—	—	50	Dyspnea and salivation.
3.30	52.8	27.1	—	—	—	—	—	—	
4.05	50.0	57.6	—	—	—	—	—	—	
4.07	—	—	—	—	—	—	—	25	Salivation continuous.
4.45	—	—	—	—	—	—	—	25	Dyspnea only brief.
4.50	53.3	53.8	—	—	—	—	—	—	
5.15	—	—	—	—	—	—	—	25	Constant salivation,
5.40	—	—	—	—	—	—	—	25	transitory dyspnea.
6.00	—	—	—	—	—	—	—	25	Depression.
6.30	—	—	—	—	—	—	—	25	Clear consciousness.
6.45	—	—	—	—	—	—	—	25	
6.50	49.1	31.9	—	—	—	—	—	—	
7.10	—	—	—	—	—	—	—	25	
7.30	—	—	—	—	—	—	—	25	
8.00	—	—	—	—	—	—	—	25	
8.20	—	—	—	—	—	—	—	25	
8.40	—	—	—	—	—	—	—	25	
9.00	—	—	—	—	—	—	—	25	Symptoms not much changed.
9.40	—	—	—	—	—	—	—	25	
10.00	36.9	57.6	112	—	.13	.012	1.5	—	Rectal temp. 39.6° C.
July 8, 1915									
a. m.									
1.00	32.5	64.3	—	—	—	—	—	—	Dog acts practically well.

TABLE 22

Dog G7-66

Time July 7, 1918	BLOOD		URINE					Injections of N/2 Butyric Acid cc.	Remarks
	Corp. Vol. %	CO <sub>2</sub> Vol. %	Vol. cc.	Nitro- Prus- side	Total N gm.	NH <sub>4</sub> - N gm.	Alka- linity (cc. N/7)		
P.M.									
3.40	41.6	55.2	—	—	—	—	—	—	No symptoms.
3.45	—	—	—	—	—	—	—	50	
3.50	42.5	65.3	—	—	—	—	—	—	
4.10	44.6	71.9	—	—	—	—	—	—	
4.50	—	—	—	—	—	—	—	25	
4.55	42.5	70.0	—	—	—	—	—	—	No dyspnea or salivation. Very slight depression. Much less intoxication than Dog G7-65.
5.20	—	—	—	—	—	—	—	25	
5.45	—	—	—	—	—	—	—	25	
6.05	—	—	—	—	—	—	—	25	
6.35	—	—	—	—	—	—	—	25	
6.50	—	—	—	—	—	—	—	25	
6.55	40.8	86.2	—	—	—	—	—	—	
7.15	—	—	—	—	—	—	—	25	
7.35	—	—	—	—	—	—	—	25	
8.05	—	—	—	—	—	—	—	25	
8.25	—	—	—	—	—	—	—	25	Beginning of muscle quiverings and dyspnea.
8.45	—	—	—	—	—	—	—	25	
9.05	—	—	—	—	—	—	—	25	Intense noisy dyspnea, 168 per minute. Distress, prostration; general spasticity and muscle twitching. Clear consciousness. Rectal temp. 42.4° C. Injected 400 cc. 0.85% NaCl into jugular. All symptoms slightly relieved.
9.45	—	—	—	—	—	—	—	25	
10.00	56.6	76.7	600	++	1.26	.036	12.6	—	
10.30	73.5	89.6	—	—	—	—	—	—	
10.35	—	—	—	—	—	—	—	—	
10.45	—	—	—	—	—	—	—	—	Convulsions and death. Intense heat inside cadaver. Autopsy negative except for venous engorgement.
July 8 A.M.									
12.50	46.6	61.4	78	+	.14	.006	1.0	—	

*Nitroprusside reactions* remained negative in the urine of the dog receiving the butyric acid solution, but were positive in the animal receiving the sodium butyrate. It is unknown whether the difference was accidental, or whether it has some connection with the long accepted clinical rule that alkali increases the excretion of acetone bodies.

The *plasma bicarbonate* was lowered only briefly by the acid injections in dog G7-65, rising to normal as the acid was rapidly burned. The alkali released by this combustion in dog G7-66 elevated the plasma bicarbonate, which apparently would have reached extreme values except for the dilution with the intravenously injected saline solution.

The *clinical effects* seemed at first more pronounced in the dog receiving the acid. But with increased dosage, the apparent protection afforded by the alkali was changed to intoxication, and death evidently resulted from alkalosis. The autopsy was negative except for engorgement of the great veins, viscera, brain and meninges. Microscopic examination of the principal viscera showed nothing additional, except slight vacuolation in the renal tubules.

TABLE 23

Dogs G7-63 and G7-65. July 11, 1918

Hour P.M.	CORPUSCLE VOL. %		PLASMA CO <sub>2</sub> VOL. %		INJECTIONS CC.	
	G7-63	G7-65	G7-63	G7-65	G7-63 Butyric acid	G7-65 Sod. butyrate
3.50	43.1	31.8	59.5	67.1	—	—
4.00	—	—	—	—	25	50
4.15	46.3	26.9	59.5	55.3	—	—
4.20	—	—	—	—	25	50
4.30	45.0	—	54.8	—	—	—
4.35	—	—	—	—	25	50
4.45	47.4	25.6	17.6	73.9	—	—
4.50	—	—	—	—	25	50
5.00	42.5	—	14.7	—	—	—
5.05	—	—	—	—	25	50
					Death	
5.15	—	—	—	—	—	50
5.20	—	24.0	—	76.7	—	—
5.25	—	—	—	—	—	50
5.30	—	—	—	—	—	50
5.40	—	24.0	—	72.9	—	—
5.45	—	—	—	—	—	25
5.50	—	—	—	—	—	25
6.00	—	22.6	—	67.2	—	—
6.25	—	—	—	—	—	50
6.30	—	20.8	—	51.9	—	—
6.35	—	—	—	—	—	50
6.40	—	—	—	—	—	50
6.45	—	18.4	—	59.5	—	—
6.50	—	—	—	—	—	50
7.00	—	—	—	—	—	25
7.15	—	22.4	—	62.4	—	—
7.25	—	—	—	—	—	Death

Dog G7 - 65, which was used for the experiment shown in table 21, ate practically nothing after that experiment, and by July 11 had lost weight down to 7 kg. On that day it was used for larger butyrate injections, in comparison with dog G7 - 63, which weighed 7 kg. without fasting. The latter dog received intravenous injections of 0.75 N butyric acid. Dog G7-65 received the same solution in twice as large dosage, but neutralized with NaOH against litmus. Table 23 shows the observed blood changes.

Nitroprusside reactions remained negative in the urine and plasma of both dogs.

The plasma bicarbonate fell rapidly as dog G7 - 63 was overwhelmed by the acid injections, and the acidity in itself was evidently the most important factor in the early death. By virtue of the protective effect of the alkali, dog G7 - 65 was able to take over five times as much butyric acid before succumbing. Neither the symptoms nor the plasma bicarbonate



analyses indicated death from alkalosis, and the essential factor seems to have been the toxicity of the butyrate itself.

The *clinical effects* in dog G7 - 63 were dyspnea, at first transitory and never extreme, and later drunkenness, passing over at the end into complete coma with absence even of the conjunctival reflex. The picture therefore bore some resemblance to diabetic coma. Dog G7 - 65 showed no symptoms from the first two injections. With the third one, there was a beginning of depression, unsteadiness and crying, which increased with the succeeding injections. After the fifth injection the animal was unable to stand and consciousness was impaired. With the later injections first semi-consciousness and then unconsciousness developed, though the conjunctival reflex was retained up to the last two or three minutes of life. Dyspnea, muscle twitching and convulsions remained absent. Heart and respiration stopped simultaneously. The picture seemed to be one of death from narcotic intoxication of the nervous system.

The gross and microscopic autopsy was negative in both animals, the chief difference being a much greater engorgement of the veins, viscera and nervous system in Dog G7 - 65 than in G7 - 63.

#### SUMMARY AND CONCLUSIONS.

1. Intoxication with acetone and the lower fatty acids is characterized by dyspnea and drunkenness passing on into coma and death. These symptoms are specific and not due to mere acidity, for they are produced by acetone and neutral salts of the acids, and furthermore a mineral acid such as hydrochloric produces a different picture with much less resemblance to diabetic coma.

2. Special mention may be made of the dyspnea, which is greatest when free acids are administered, but nevertheless is produced markedly by acetone or the sodium salts of the acids. Acetone injections are valuable in this connection, since the reduction of plasma bicarbonate produced by them cannot be due to acidity, unless there is a secondary excess formation of acids in the body. Though such a formation is possible, it seems plausible that the alteration of the respiratory function constitutes at least one factor. It is thus furthermore probable that intoxication with acetone substances and diabetic coma are conditions in which the plasma bicarbonate is not an accurate measure of the alkali reserve of the body, though it was not possible to continue the experiments to a demonstration of this point. On the other hand both the dyspnea and the reduction of plasma bicarbonate are ordinarily less with the injections than with diabetic coma, so that some degree of true acidosis must be assumed as necessary to complete the picture of typical coma.

3. The individual acids obviously differ in toxicity. It was not possible to carry out the exact comparisons of toxicity which were contemplated, but there was a general impression in favor of Hurtley's contention that acetoacetic acid is much more toxic than  $\beta$ -oxybutyric. The toxicity of butyric acid is also high. It may therefore be permissible from one standpoint to regard the formation of  $\beta$ -oxybutyric from either butyric or acetoacetic acid as a partial distoxication.

4. Positive nitroprusside reactions in urine or blood were obtained frequently after butyric and sometimes after  $\beta$ -oxybutyric acid injections. Any traces of acetoacetic thus indicated do not stand opposed to the doctrine that  $\beta$ -oxybutyric gives rise to no large quantities of acetoacetic acid. But the slightrness of the reactions also indicates only a small formation of acetoacetic from butyric acid in normal animals. Though this test does not furnish a measure of  $\beta$ -oxybutyric formation, ketosis of a degree sufficient to cause intoxication or coma in man or animals is always accompanied by heavy nitroprusside reactions in urine or plasma. From this it may be deduced that butyric acid causes strictly a butyric acid intoxication, which may bear some symptomatic resemblance to diabetic ketosis and coma but is by no means identical.

5. It was unfortunately impossible to carry out the analyses of blood and tissues which were contemplated, and thus learn what quantities of the different acids accompany lethal and sublethal intoxications, and how these compare with the concentrations found in clinical coma and in the various forms of ketosis in animals. One of the chief objections to the theory of diabetic coma as a pure intoxication by one or all of the acetone substances lies in the fact, indicated by the scanty clinical evidence available, that these substances differ greatly in their quantities in blood and urine in fatal cases, and may be higher some days before death than at death.<sup>27</sup> In the injection experiments, the nitroprusside reactions demonstrate that acetone persists for surprisingly long periods—perhaps a whole day—in the blood after a single dose. Nevertheless the quantity in the body immediately after a subcutaneous injection is probably much greater than at the time of death. After certain doses of acetoacetic acid, delayed death may occur after nitroprusside tests have become negative in the blood, and the tissues have no acetoacetic



odor. Similarly, when death occurs a considerable time after a butyric acid injection, there may be no butyric odor to the blood or tissues. It thus seems probable that substances such as the ketones belong to the group of poisons which can cause death even after the substance itself has been destroyed. An explanation may thus be afforded not only for the variable quantities of the acids in clinical cases of coma, but also for the fact that dogs may die of ketosis even when the ketones have greatly diminished or disappeared before death, and furthermore for the recent observations that diabetics in the last stages of coma are not saved by insulin even though acidosis and ketosis be completely cleared up.

6. Autopsies are essentially negative with the artificial intoxications, just as with spontaneous ketosis. The extreme feebleness of the circulation is common to the two conditions, but the engorgement of the venous system at death is peculiar to the injection experiments.

7. In general, animals predisposed to ketosis (young fasting puppies, phlorizinized and depancreatized dogs) are more susceptible than others to these injections, in the sense both of a greater ketosis from the fatty acids, and also more severe symptoms and injury. These results in the case of acetoacetic and  $\beta$ -oxybutyric acid are logically associated with the impaired power of metabolizing these substances. But if it be correct that no large quantities of these two acids accumulate in the body following the injection of butyric and other higher acids, the intoxication being chiefly due directly to these other acids, then it must follow that an impaired ability to metabolize these other acids is also demonstrated under these conditions. Pushed to its furthest limit, such observations may suggest the speculation that carbohydrate is required for the normal metabolism of fat not merely at the ketone stage but also at higher stages.

8. All acids are more toxic than their neutral sodium salts, up to a certain limit. The plasma bicarbonate analyses make it obvious that the combustion of the organic acid often leaves an excess of alkali in the blood. With a suitable arrangement as respects quantity and timing of doses, it is demonstrable that animals may survive a certain amount of acid but die from the equivalent in the form of the sodium salt, on account of alkalosis. It is important that the dyspnea and other symptoms of alkalosis



be not confused with the effects of the acid itself. There is probably no very important clinical lesson, for even excessive doses of alkali are usually safely disposed of by the kidneys. Only when there is renal injury with partial anuria may the neutralization of an acidosis perhaps be turned into a dangerous alkalosis when the organic acids have been burned.

9. Hyperglycemia, sometimes to the point of slight glycosuria, is among the incidental effects of large doses of the fatty acids. It is not an effect of acid reaction, because it also results from acetone and the neutral salts of the acids. It is probably to be classed with the hyperglycemia from ether and all sorts of asphyxial agencies. If further experiments shall show that the acid injections also counteract the effects of insulin, the observation will furnish a further analogy between the artificial intoxication and diabetic coma, and an explanation of the enormous doses of insulin required to produce results in severe ketosis. On the other hand, lack of such a resemblance with respect to insulin must appear as an important difference between diabetic coma and ketone intoxication.

10. On the whole the investigation favors the view that specific intoxication with the acetone substances, together with an element of true acidosis, is the chief known cause of diabetic coma. The variable picture of coma, with sometimes stupor and sometimes dyspnea predominating, may be explained by the variations in these two factors.

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## ERRATA

In the paper of Gauss, this JOURNAL, Vol. 4, the following corrections should be made:

Table I (Page C) log for 25.5 (temp) and 646 mm. (bar. press.) is given 37191; it should be 87191.

log for 20.0 (temp) and 655 mm. (bar. press.) is given 80142; it should be 89142.

In the paper of Höst, the following corrections should be made:

Page 322, 16th line from below, "After filtration and treatment of the filtrate from HCl and Zn, about 5 cc. was taken" should read "After filtration and treatment of the filtrate with HCl and Zn with subsequent filtration, about 5 cc. of this filtrate was taken".

Page 325, 8th line from above, add "Urine No. 6 same as No. 5".

Page 328, 5th line from above, "the colorimetric method 44" should read "the colorimetric method 45".

Page 357, Experiment 86, the figures printed are wrong; they should read:

50	0.40	20	90
51	0.38	19	129
40	0.40	16	158
32	0.42	13	158
30	0.40	12	156
			92
			63
			63
			67



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## ANNOUNCEMENT

*A plan existed whereby a large European work would have occupied this entire JOURNAL for 1924, and thus at one stroke publication would have been brought up to date for 1925. Long delays have disappointed this expectation, and the JOURNAL therefore finds itself one year behind time. On the other hand the number of manuscripts received is increasing so that publication can now be hastened. The JOURNAL will probably appear in quarterly form until this material is disposed of and publication brought up to date. After that, the intervals of publication will be governed by the material received, but it appears probable that the monthly basis can be resumed.*

THE EDITOR.

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